STRUCTURAL STUDIES OF CHEMICAL CONSTITUENTS OF PLANTS OF BUNDELKHAND REGION

A THESIS

submitted for the degree of Doctor of Philosphy in Chemistry of Bundelkhand university

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GERTALIA (SALE)

"STRUCTURAL STUDIES OF SOME CHEMICAL CONSTITUENTS OF PLANTS OF BUNDELKHAND REGION" submitted by (Rm.) Shobha Devi fulfills all the requirements of Ph. D. degree of Bundalkhand University, Jhanel. She reported her own research work with the investigation of a new Polysaccharide, carried out under my supervision and guidance. She did her research work regularly and more than 200 days as desired by University Statute, at the research laboratory of Chemistry Department, Dayanand Vedic (Postgraduate) College, Grai of Bundelkhand University, JHANSE (U.P.).

January 28 ,1983.

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PREFACE

The dissertation entitled, "STRUCTURAL STUDIES OF CHERTICAL CONSTITUENTS OF PLANTS OF SUMPELSUAND REGION", deals with the isolation and chemical examination of polysaccharide from the seeds of <u>Sizyphus runosa</u> and fruits of <u>Musasapiantum</u>, some constituents from the fruits of <u>Gardenia gumnifera</u>. The thesis has been divided into four chapters.

The chapter I is of introductory nature and described the wide importance of natural products and a brief account of different classes of compounds, i.e. polysaccharide, flavanoids and anthocyanins.

The chapter II deals with the isolation and structural elucidation of neutral water soluble polysaccharide from the seeds of <u>Zizyphus rucosa</u>.

The chapter III describes the isolation and structural elucidation of a water soluble neutral polysaccharide from the fruits of Musa sapientum-

The chapter IV is divided into three Sections (A), (B) and (C) deals with the isolation and elucidation of chemical structures of two flavonoids and a anthocyanin from the fruits of <u>Gardenia gumnifera</u>.

A brief review of uptodate literature on chemical examination of selected plants, has been described respectively in each concerned chapters.

The work represented in the thesis has been earried out in the chemical laboratories of Dayanand Wedic Post-graduate College, QRAI, (Bundelkhand), under the supervision of Dr. G_*S_* , Niganjan, D. Phil., $F_*I_*C_*S_*$, Department of Chemistry, Dayanand Vedic (P_*G) College, QRAI (Bundelkhand).

A brief summary of the entire work has been submitted separately alongwith the thesis, according to the requirement of ordinances for Ph. D. degree of Bundelkhand University.

ACONOMINE DOMENTS

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The achievement of the goal would have been a hard nut to crack without the constant encouragement and help given by Dr. Y.S. Srivastava, Dr. S.K.Katiyar, Dr. B.C. Sikrosia and Dr. R.K. Gapta who provided valuable guidence, suggestions and inspiration during my research parios.

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I am obliged to the authorities of C.D.R.I., Lucknew and I.I. T. Kanpur for valuable assistance in recording IR and U.V. spectra of the compounds and giving all facilities to consult important and valuable literature during the research period.

I will be failing in my duty if I do not record my sincere and heartfelt gratitudes to my parents and all family members for their co-operation, inspiration, guidence and seal shown/given without whom the present important work could not have been acomplished successfully.

I also offer my entreme gratitude and most sincere thanks to Smt. Dr. G.S. Niranjan, for all the affections showred on me during the period.

Last but not least I also wish to express my profound gratitude and thanks to Sri A.K. Gupta, for his encouragement, co-operation and guidence given to me throughout the course of the research work which has been invaluable to me and which I can never forget.

CHEMICAL LABORATORISS, D.V. (P.G.) COLLEGE, ORAI - 285 001. Shobha Deni (SHOBHA DEVI)

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CEAPTER - 1

Nachrie

There are still a large number of plants which have not been investigated for their active principles among the plant kingdom. The promiseous centre of nature awaits for plant chemist for their valuable investigations, may serve the human sufferings.

The plant products obtained from the plants are classified into following groups: (1) alkaloids, (2) glycoside, (3) saponing (4) tempenes and tempenoids, (5) bitter principles, (6) escentic cits, (7) fatty cits and wates, (6) lactones, (9) reside and tempine, (10) steroids and phyto steroides, (11) phenolic compounds, (12) organic acids, (13) hydrocarbons, (14) dust and muctiage, (15) sugars.

This classification is not rigid, in the sames that one compound may be said to belong to more than one groups according to its molecular structure various chamical constituents obtains from the plants are classified into many groups. A brief account the review on the classes of compounds investigated from the plants, which have been incorporated in the present thesis is given below :

- I.2 Polysaccharides.
- 1.3 Flavonoida.
- I.4 Anthocyanin.

1.2 POMSACCHARIDIS

Polysaccharides are components of almost all living organism. They are most abundant in the higher orders of land plants and sea weeds where they constitute approximately three

quarterts of the dry weight. They are present in fungi excelleton of insect and crustaceuns, in the capsules of microorganism, in cartiláge, in animal joint fluids etc.

Polysaccharides are macromolecular compounds composed of several monosaccharide units, usually linked through oxygen to give complex composition. They are regarded as condensation polymers of monosaccharides resulting from the formation of glyconidic linkage by elimation of water. They are hydrophillic colloids of high molecular weight, some completely soluble in water, other swell and absorb considerable amount of water without dissolving.

ques and mucilages are complicated polymeral and differ in the respect that the former are characterized as plants exudate-s while the latter are isolated from various plant organs by extraction with water. Plant gums and mucilages have been known and in the since very early times, reference being made to them in the 'Sible'; and they seem to have been of commercial value for several thousand years, especially in India, Asia, Africa, Australia and China.

Polysaccharides act deserving roles in the physiology of plants, animals and microorganism as surface material and regarded as food reservers in much the same manner as starch in many plants and glycogen in animals or as agent for holding water. The plant is believed to synthesize the gum scudate in order to seal off the infected section of the plant and prevent further invasion of the tissue. There is no concept as to the origin of gum scudates, whatever the stact origin and more of formation of the gums may be, it is

reasonable to believe that gum exudates are formed by some type of ensymmtic polymerisation and not by direct polymerissation.

Gums and mucilages are used in wide range of industries like cosmetics 11,12,13, pharmacy 14,15, tentiles 16,17, adhesives 18, food products 19,20,21, paper 22,23, and in many other fields.

A polysaccharide is isolated from the plant by extraction with cold or hot water, water containing a little acetic acid and the precipitation of the soluble portion with the escess of otherol. The polysaccharide is purified to remove the inorganic ions and protenious impurities by repeated precipitation with ethanol from acidified aquous solution.

The homogeneity of the polysaccharide is checked by fraction precipitation ²⁵, some electrophoresis ^{26,27} and acetylation and descetylation ²⁸. A mixture of polysaccharide can be separated over a cellulose column ^{29,30}, while the acidic polysaccharides may be fractionated as their completes ³¹. Ion exchange column ³² are also used effectively for the fractionation but the methylated gums are separated over alumina ³³. Electrophoretic separation of polysaccharides have been achieved mainly in borate buffer ^{34,15}, but acetate buffer ^{36,37} and citrate buffer ³⁷ have also been used. With the help of membranes or filters of desired porosity ³⁸ polysaccharides may be fractionate the unwanted polysaccharides of the mixture may be destroyed with specific enzymes ³⁹ followed by denaturation of enzyme with heat, alkali and alcohal. The fractionation of polysaccharides may also be achieved by gel filtration ⁴⁸ and molecular sieve ⁴¹.

The purified polysaccharide is subjected to preliminary determination of lignin, ash content, methodyl, acetyl, primary hydroxyl and earbonyl groups and they are estimated after the detection of nitrogen, sulphur, phosphorus and halogens which may be present in the polysaccharide.

The optical rotation of the polysaccharide is measured by means of usual polarimeter are photoelectric spectropolarimeters. The configuration of glycosidic linkage in oligosaccharides can be correlated to optical rotatory power by applying Midson's rule of isorotation.

The molecular weight of the polysaccharide having terminal reducing group can be determined by estimating it with c¹⁴ labelled sodium cyanide⁴⁵, sodium hypotodite⁴⁶, ferricyanide⁴⁷, and periodate oxidation studies⁴⁸. Physical methods like viscosity⁴⁹, light scattering⁵⁰, esmotic pressure⁵¹ are also used to determined the molecular weight of the polysaccharide.

The hydrolysis of the polysaccharide with mineral acids under different conditions provides information regarding the nature of linkages present between sugar moisties. The complete acid hydrolysis of the polysaccharide results in the liberation of monosaccharides which can be separated by paper 52 or column chromatographic 3 techniques. They are identified by their Rg values, co-chromatography with authentic samples, malting points and by preparing their crystalline derivatives. Partial acidic hydrolysis with dilute mineral acids (0.01 - 0.10) results in degradation of the polysaccharide into less complicated molecules which can easily be identified. Gligosaccharides, chtained by partial hydrolysis, can be separated by paper chromatography

and their structure is determined by the usual process of methylation, followed by the hydrolysis and identification of methylated sugars, periodate oxidation and emzymic hydrolysis. Insymic degradation ⁵⁴ provides various information about the polysaccharide. The sugar may be quantitatively estimated by microvolumetric method, spectrophotometric method or colorimetric method. Recently an extensive use of gas liquid partition chromatography ^{55,56} in the separation and estimation of sugars has been reported.

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The polysaccharide is subjected to periodate oxidation to obtain the information regarding the nature of end groups and types of glycosidic linkage Sy present. It has been observed that the 1,2 -diol groups in $1\rightarrow 2$ or $1\rightarrow 4$ linked and 1,2,3-triol groups in the $1\rightarrow 6$ linked anhydrohexose units are exidised by one and two moles of periodate respectively. Liberating one mole of formic acid but the units having $1\rightarrow 3$ linkages with no 1,2 - diol system are not effected. Thus by determining the consumption of periodate and amount of formic acid liberated, various informations regarding the structure may be obtained.

The methylation studies serve the valuable information regarding the types of linkages between sugar moieties in a polysaccharide. The method consists in the methylation of the polysaccharide followed by hydrolysis to give methylated sugars. The nature and the quantitative determination of the mathylated sugars provide information on the relative proportions of non-reducing end groups, the degree of branching, the type of interchain linkages and the nature of the main chain linkages

in the polyenecharide. Nethylation is usually carried out by means of Haworth's method⁵⁸ followed by Purdie's method⁵⁹. The methylated product is hydrolysed in two steps, first the methanolic hydrogenchloride⁶⁰ or with 85 - 98% formic acid⁶¹ and finally with the mineral acids. The methylated sugars are separated on paper and hedentified by their *PW⁶² values, optical rotations and melting points of their crystalline derivatives. The methylated sugars are quantitatively estimated by titrating them with alkaline hypoiodite or by colorimetric method. Those polysaccharides which are soluble in dimethyl sulphoxide, may be very efficiently methylated⁶³ in fewer steps by using methyl iodide and aflyer on-ide.

In the present thesis, the chemical examination of two complex water soluble polysaccharides, first (isolated from the seeds of <u>Zizyphus rugosa</u>) and second (isolated from the fruits of <u>Musa sapientum</u>) have been described in chapter II and chapter III respectively.

1.3 FLAVONOTOES

The said

Playonoids covers a largest group of naturally occurring oxyheterocyclic pigments. They includes chalcones, dihydrochalcones, aurones, flavones, flavonois, isoflavones, anthocyanins and leucoan-thocyanidins. In these two benzene ring are linked by a propage bridge (C - C - C - C - C) except in isoflavones in which the structure based upon C - C - C - C - C. Flavonoids are present in plants as yallow

pigments. These are found in the free state as Well as in the form of glycosides, containing either sugars or more than one hydroxyl group or disaccharide (bioside) and trisaccharides.

It is supposed that flavones protect plants from harmful ultraviolet radistions or from loss of important materials by autocaidation and one is tempted to believe physiological functions of the flavonoid pigments based upon their colours are related to the role of flowers in reproduction ⁶⁴. These compounds were found to be great medicinal importance as bacteriostatic ⁶⁵ and insecticidal etc.

1.1.1 PLAYOUS AND PLAYONOLS

The flavones and flavonols are naturally colouring matters. Their structure is based on that of 2 - ihenyl + 4 + Chromone. The basic unit of flavone and flavonols is $\sqrt{-pyrone}$ which is present as benso $-\sqrt{-pyrone}$ (chromone). The basic $-\sqrt{-pyrone}$ is substituted by a phenyl group in position -2 to give the first member of the class of flavones when a hydrogen atom on C_3 in the $\sqrt{-pyrone}$ ring of flavone is replaced by a hydroxyl group, 3 - hydroxy flavone or flavonol is produced. The basic skeleton of flavone and flavonol may be represented as :

Flavone skaletor

The basic ekeleton of flavonol may be represented as under a

Plavenol steleton

The flavones which are also known as anthoganthing and widely distributed yellow plant pigments. They occurs either in the free state or as glycosides or associated with tenning. They are also occur as colougless glycosides in the white corollas of several flowers which on treatment with amonda vapour turns yellow (as the colougless precursors are converted into flavones).

shortly, the flavones and flavonols occurs as glycosides and on hydrolysis they yield the sugar moieties and a sugar free portion (a glycone) known as flavone or flavonol as the case may be. The position occupied by a sugar unit in glycosidic linkage, plays an important part due to which a glycoside exhibits difference in properties as solubility and capacity to form complemes with metals. Unlike anthocyanins in which the sugar residue is usually present at position 3 and 5, the sugar moiety in flavone and flavonols is generally attached to a hydroxyl group at position 3 or 7.

These compounds have been found to be highly physiologically active. The flavonol glycoside rutin has been described for its therapeutic properties. The insecticidal action of polyhydroxy flavones and their ethers and the action of

flavones on isolated ensyme system⁶⁶ have been studied.

The author has been able to isolate a flavone compound and a flavonol glycoside from the seeds of <u>Gardenia gumnifers</u>. The chemical study of these colouring substances has been described in chapter IV of the thesis.

ANTHOCYANTING

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Anthocyanins comprises of a group of glycosidic pigments responsible for various colours, particularly red, violet, and blue, in flowers, fruits, (Begries), stems, leaves and roots of the plants. They are soluble in water and generally occur in the aquous cell-sap. Anthocyanins are amphotoric in nature, their acid salts are red, alkali, salts are blue and free anthocyanins (or neutral) are violet. The different shades of the flowers are due to the presence of some anthocyanins in different media (acidic, alkaline or neutral).

Phenyl bensopyrylium chloride as the parent compound. Anthecyanins and anthocyanidines are derivatives of 3:5:7.

trihydroxy flavylium chloride. The various pigments (anthecyanins and anthocyanidins) are differ in the number, nature
and position of other hydroxyl groups, methoxy groups and sugar
residue. The basic skeletom of anthocyanidin is represented
as follows:

Flavylium chloride

The basic skeleton of anthocyanidin is represented as under in continuation

+ + 4 |

3:5:7 - trihydroxy flavylium chloride

Many flowers that are first colourless, are known to develop colour rapidly and the colourless plant constituents which can be converted into anthocyanidins by boiling with aquous or alcohile hydrochloric acid, are known as leucoanthocyanins, anthocyanins on hydrolysis with mineral acids or ensyme break into the sugar moiety and sugar free pigment, called anthocyanidine or aglycome. The common sugars found in anthocyanins are glucose, galactose, rhamnose.

The colour variation is due to the presence in the cell vacuole of a range of different anthocyanine. Flower colour variaties arise either by spontaneous generalation 67 within a single species or when two closely related species are hypridised.

The anthocyanins may be present in any part of the plant from the root tip to the flower stigms, but intense permanent pigmantation by anthocyanins are generally confined to petal or fruit tissue . Deaply coloured flowers may be born on plants with essentially anthocyanin free stem and leaves.

- 1. Stanl, S.; Chemiker. 2kg., 82 , 323 (1958).
- 2. Fieser, M. and Fieser, L.R.; J. org. chem. , 13
- 3. Dobriner, L.I.; J. Amer. chem. SCC., 22, 3215 (1951).
- 4. Fuson, A. ; J. Amer. Chem SQC., 74 , 5206 (1952).
- 5. Royl. Histler and Charles Smart; 'Polysaccharide Chamistry', P. No (1-18), (1953), New York.
- 6. Montemartini, L. ; Lavori ist. botan., 5 , 45 (1934) Palarmo.
- 7. MeMair, J.B., Amer. J. Botany, 19, 168 (1932).
- 8. Frank, H.B. p Ann. Agronom., 17, 86 (1885); Arit. chem. Abstracts, 48, 684 (1885).
- 9. Brooks, P.T. , New Phytologist, 27 , 85 (1925).
- 11 Hilfer, H. ; Drug and Cosmetic Ind., 67 , 774 (1950).
- 12 Hedgrove, H.B. p Ind. Chemist. , 16 , 145 (1940).
- 13 Anderson, E. , J. Chem. 2 , 853 (1932), Ed.
- 14 Sehenk, G.; Med. Monateschr., <u>3</u>, 700 (1949); Chem. Abstr., <u>44</u> , 1223 (1950).
- 15 Hanslik, P.J., De Ede, F., Empey, L. W. and Pagg., W.H.; J. Pharmacol . . 32 , 273 (1927).
- 16 Pinel, A. ; Brit. Pet. , 522 , 815 (1940).
- 17 Hersog, R. O. and Meler, A. / U.S. Pat. . 1 . 161. 545 (1915).

177

- 18. Outman, A.E. ; Colloid. chem. , 6 , 248 (1946).
- 19. Pozrin, P.H. , Pr. Pat. , 860 , 210 (1941).
- 20. Pyenson, H. and Dahle, C.D. ; J. Dairy Sci. , 21, 169 (1938).
- 21. Stall, A.C. , Pood Research, 17 , 278 (1952).
- 22. Swanson, J.W. 1 Tappi. 33 . 77. 451 (1950).
- 23. Osawa, To ; J. Chem. Ind. <u>25</u> , 309 (1922) ,(Japan),; chem. Abstr ; <u>16</u> , 4086 (1922).
- 24. Ball, $D ext{-}J$, and Young, $F ext{-}G$. # Biochem. $J ext{-} ext{-} ext{28} ext{-}$ 882 (1934).
- 25. 0° Sullivan, C. ; J. chem. SCC., 45 , 41 (1884);
 79 , 1169 (1901).
- 26. Jouhert, F.J. ; J. South African Chem. Instt., 7 (2),
- 27. Presce, I. A. and Phibkirk, R. ; cham and Ind. , 257 (1955).
- 28. Haworth, W.M., Hirst, E.L. and Smith, F. , J. cham. Soc., 1914 (1939).
- 29. Aming, B.S. , J. Chem. SCC. , 282 (1955).

T.

- 30. Berenson, G-5. ; Biochem. Biophys. Acta. 22 . 176 (1958).
- 31. Antonopoules, C.A. Borelius, E. Gadell, S.,
 Hamnostrom, B. and Scott, J.E., Siochem. Biophys.
 Acta, S4 , 123 (1961).
- 32. Naukom, H., Davel, H., Heri, H.J. and Munding, W. ;
 Nelv. chim. Acta, <u>43</u> , 64 (1960).

33. Jones, J.K. N. 1 J. cham. SOC. , 333 (1944).

1.6

* 1.3

- 34. Poster, A.B., 'Advances in Carbohydrate chemistry',
 12 , 81 (1957).
- 35. Fuller, K_7M_7 and Hogthcote, D_7M_7 ; Biochem. J., 64 , 657 (1956).
- 36. Lawis , B,A, and Smith , P. ; J. Amer. cham. SQC. , 29 , 3929 (1957).
- 37. Brookhart, J. H. & J. chromatog. , 20 , 191 (1965).
- 38. Mould , D.L. and Synge, R.L. ; Analyst, 77 , 964(1952).
- 39. Adams, M., Richtsyer, N.K. and Hudson, C.S., J. Amar. chem. SCC., 65, 136 (1943).
- 40. (a) Flodin, P. ; "Destran Gals and their application in Gal filtration . Ph.D. , Dissertation,"

 Upsala University, Uppala (1962).
 - (b) Nordin, P. ; Arch. Biochem. Biophys., 99 , 101 (1962).
- 41. (a) Flodin, P. and Porath, J. s "Chromatography",
 E. Heftman edition, Reinhold Pub. Corp., N.Y.,
 P. 328 (1961).
 - (b) Jones, J.K. N., Wall, R.A. and Pittel, A.O., Canad, J. chem., 38, 2285 (1960).
- 42. Kaend, W. ; Starke, 14 , 246 (1962).
- 43. Klyne, W.; "Advances in Organic chemistry", 1, 650 (1959).
- 44. Hudson, C.S. ; J. Amer. chem. SQC., 31 , 66 (1909).
- 45. Moyer, J.D. and Isbell, H.S., Anal Chem., 30 1975 (1958).
- 46. Chanda, 5.K., Hirst, E.L., Jones, J.K.N., and Peopelval, E. G.V.: J. cham. 500., 1289 (1950).

47. Nussembaum, S. and Hassid, N.Z. ; Anal. Chem., 24. Sol (1952).

. 1

. 32

. 04

- 48. Whalam, W.J., ; Mathods in Carbohydrate chemistry',
 Bi. by R.J., Whistler, 4 , 72 (1964).
- 49. Cowie, J.M.G. and Greenwood, C.T., J. Amer. 5 CC., 2962 (1957).
- 50. Everett, W.W. and Poster, J. ; J. Amer. chem. SQC., 81 , 3439 (1959).
- 51. Jorgenson, B.B. and Jorgenson, C.B., Acta, Chem. Scand., 14 , 213 (1960).
- 52 (a) Bourhe, E.J., Lees, E.M. and Weigel, H., J.
 Chromatog., 11 , 253 (1962).
 - (b) Hay. G.W., Lawis, B.A. and Smith, F.J., Chromatog,
- 53. Binklay, N_1N_2 and Alternburg, N_2P_2 ; Intern. Sugar.

 J. : 66 . 217 (1964).
- 54. Araki, C, and Araki, K.; Bull. chem. SOC. (Japan),
 29. 339 (1956); chem Abstr., 51. 3465 (1957).
- 55.(1)Me Innes, A. G., Ball, D.H., Cooper, P.P. and Bishop, C.T.: J. Chromatog., 1 , 556 (1958).
- 56.(ii)mishop, C. T. and Copper, F. P. : Canad. J. Chem., 38 , 388 (1960).
- 56. Kircher, H. W. : Anal. Chem., 32 1103 (1960).
- 57. Hay, G. W. Lewis, B. A. and Smith, F. : "Mathods in Carbohydrate Chemistry", 1, 357 (1965).
- 58. Parikh, V. M., Ingle, T. R. and Shide, B. V. s J. Indian Chem. Soc., 35, 125 (1958).
- 59. Jones, J.K. N. : J. Cham. Soc., 1055 (1947).

60. Hirst, E. L., Hough, L. and Jones, J. K. N. s J. C hem. Soc., 928 (1949).

. 19

- 61. Andger, P. Hough, L. and Jones, J.K.W.; J. Chem. Soc., 3393 (1952): Doid., 2744 (1952).
- 62. Smith, P. and Montgomery, R. : "The Chemistry of Plant Gums and Mucilage's, American Chemical Society Mono-graph series, Reinhold Pub. Corp., N.Y., p. 226 (1959).
- 63. Srivastava, H.C. s Tetrahedron Letters, 27, 1869-73
- 64. Blank, F. : Botan. Rev., 13 , 241 317 (1947).
- 65. Blank, F. : and Suter, R. : Experimentia . 4. 72-73 (1948).
- 66. Cars-Coke, S. and Plaza Dales Rayes, M.;

 (a) Bol. Soc. Biol. Sentiage Chile, 4, 105-7 (1947).

 (b) Bull. Soc. Chim. Biol., 29 573-82 (1947).

CHAPTER . II

A NEW WATER SOLUBLE NEUTRAL POLESACCHAREDE

PROM THE SEEDS OF

ziemprus ruggia

II.1. The present Chapter describes the isolation and structural elucidation of a water soluble neutral polysaccharide from the seeds of <u>Sizyphus ruguma</u>.

The plant <u>Risphus rusous</u> Lam. commonly known as 'Mer'. This plant belongs to the family Whammaceas¹, a straggling evergreen shrub eften climbing or occasionally a small tree. Young branches, inflorensence, prickles and under side of the leaves usually clothed with dense rusty coloured tomentum. Prickles broad based, strong and hooked mostly solitary. Leaves variable, 2 - 5 inches in long, ovate, or elliptic from an oblique after cordate base. Nain nerves are prominent. Flowers long peduncled, stillary and terminal cymes, forming on the usually leafless branches. long terminal panicles, calyx, pubescent, inside. Drups & +/3 inches long, glabose or obovoid, 1 celled, 1 - seeded with very thin crustaceas stone. Flowering in March and April and fruit ripens during rainy season.

Indigenous and naturalized throughout India. Wild and cultivated in sub- Himalayan tract C & S. India to Ceylon, also in Burma, Dehradun, Bundelkhand, Rohilkhand, Gorakhpur.

The fruit is eaten and branches are lopped and fodderFlowers used for the remedy in Manerrhagia throughout India.
The use of Egyption wood in ancient civilization is reviewed,
and the abilities of many these species to have resisted
termite attack was studied. This specimen is 3000 - 4000
Years old.

The oldest Byyptian wood belongs to the genus Bisyphus .

The work done in the past years on this genus was surveyed and the details of it are given in the tabular form.

II.2. The Brief Review of Chemical Examination of this plant in the Literature is Described as given below :

Cara		Flant species	Constituents	7050	Paramaaa
1.	Sisyphaes		Vitamin C Content		(2036)9
2.	21.37phus		Anthraquinone derivet ive		(1959) ³
	Jujuba (Florida grown)		Carotana and ascorbic acid content		(1946) ⁵
4.	Jujuba (Korea)		lysine, aspartic acid, glycine, aspary; glutamic acid & galactose		(1949)*
5.	Zizyphuo	Talani (Balkat) (Balco)	Lignin and -callulose	Wood (Philis- ppine)	
6.	215772000	Spina Christi	Tannin	and:	(1939)
7 .	212yphus		Three Autin Protectors	Skam goll tiesus	(1980)
8.	Zizyphus		Spinisin and its acyleted degivetives	3.00	(1980) ³⁰
9.		Jacserio	Detergent analysis		(1951.) ¹¹
0.	Sinyohus	Jacosio	Justic acid	Back	(1957)12
1.	zizyphua	Mylophora	acid	Bark & Nood	(1961.) ¹³
2.	31zyphus	Miobhans	Tannins & oleanolie acid	Pruite	(1963) ¹⁴
.3.	Mayphus	Nylophyrus	(-)-Leucoanth- ocyania	Pruits	(1968) ₇₂

Gen	21.	ant spacias	Constituents	Parts	References
14.	2Lay shue	Cenoplia	The same of the sa	Root à Bark	(1963)**
15.	Zisyphue	Cenopi ia	Constitution of	Root 6	(1969) ¹⁷
16.	Zizyphue	Maurit lana	Two paptide alkaloids Mauritine (A) & Mauritine (B)	***	(1972) ¹⁸
17.	Stayolms	Murit lana	Zimogenin (a new Sapogenin)	•	(1979) ¹⁹
10.	zisyphue	Pructus			
19.	Zisyphus	Fructus	Zizyphus sapo- nins I,XI,IXX & jujubaside B and jujubogenin	Davidse	(1981) ²¹
20.	Zisyphus	Nummalaria	Na. K. Ca. Ng. Pe. Al. Cu. and In trace mineral constituents.		(1970) ²²
21.	Zizypime	Vulgaris			(2934) ²³
22.	Zisyphus	Vulgaris	Structure of Spinosin (flavone C glycoside)		(1979) ²⁴
23.	Zizyphuo	Vulgaris	Spinosin		(1979)25
	Ziayphus	Vulgaris	A new saponin		(1981)26
	Zisyphus	Vulgarie	Anaesthet ics	Lasves	
	Zisyphus	그는 하고를 하면 해가 되었다.	Chinese drug (extracted oil 89.16% fatty acids of which 90.75% are unsate (Palmitic acid	eaade	

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Gent		Plant species	Constituents Parts References
			& Phytosterol) including oleic. and β -Linoleic acids.
27.	Zisyphus	Wilgaris	Betulinic acid Seeds (1946) ²⁹
28.	Zisyphus	Jujuba	Leucocyanidin, Bark & (1961) ¹³ Leucopelargenidin, Wood and Betulinic & ceanothic acide
29.	Eisyphus	Jujuba	Ceryl alcohel, Leaves (1956) ³⁰ Alkaloids, Protopine and Besbering
30.	Zizyphuo	Jujuba	Rut in Leaves (1968) ³¹
	Zisyphas		Pive alkaloide Leaves (1978) ³² of 13 membered cyclopeytide alkaloidal ring structure.
32.	Zisyphus	Jujuba	Tannins, Fruits (1968) ³³ anthreglucosides, & seponins, Leaves Alkaloids, coumarins, anthocyanins, glavone glucoside, and sucilage.
33.	Zizyphus	Jujuba	Carbohydrates, Fruits (1969) ³⁴ carotene, tennins, flavone glycosides, sapomins, lipids, resins, and mucilage.
ж.	Zizyphus	Jujuba	Cyclic adenosin Fruits (1980) ⁹¹
	Zizyphus	Jujuba	Oil, contained Seeds (1953) ³⁵ olaie, linoleie, arachidic and behanic acids.
36.	Zisyphus	Jujuba	Resential animo Seeds (1969) ³⁶ acid contents.

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CODI		Plant species	Constituents	REC	Rafe Fances
37 .	Zizyphus	Jujuba	Sapagenin (Shelin lactone)	Spods	(1970) ³⁷
38.	31.577 hus	Jujuba	Saponin (Juju boside B _e structure		(1976) ³⁸
			elucidation by carbon-13 nuclear magnetic resonance)		
39.	Zisyphus	Jujuba	Autine	ando sp-	· (1969) ³⁹
40.	Sing thus	Motumdifolia	oil content, protein and fatty acids	Sands	(1979)40
41.	Zizyphus	Sat Iva	Sativanine A & B (cyclopaptide alkaloids).	Bark	(1979) ⁴¹
42.	Zizyphus		Starch moisture 68.4%, ash contest 1.65%, starch prepared 1.29%	Seedis	(1949) ⁴²

A number of chemical compounds have been already described in the above literature, but no attempt has been made for the isolation and structural elucidation of polysecharides of <u>lizychus ruposa</u>. Because of the medicinal and industrial values of the plant, it was considered worthwhile to isolate and establish the structure of the polysecharide isolated from the seeds of <u>L. ruposa</u>.

II.3. STRUCTURAL ELUCIDATION OF NEUTRAL HATER SOLUBLE POLISACCHARIDE.

results and discussion

The polysachharide was isolated from the defatted seeds of 2. rugosa, extracted with water (1% acetic acid) and precipitated with ethanol. The polysaccharide was purified by repeated precipitation with ethanol to get a white fibrous mucilage with minimum ash content (0.8%). The homogeneity of the polysaccharide was checked by s

- (i) Fractional precipitation.
- (11) Zone electrophoresis.
- (111) Acetylation and deacetylation.

The polysaccharide was dissolved in water and separated into two fractions by fraction precipition with different volumes of ethanol. Both the samples were analysed quantitatively by the method of Hirst and Jones 43. The results were essentially identical showing the homogeneity of the polysaccharide.

The polysaccharide was acetylated with aceticanhydride by the usual method to give the acetylated product, $\left[\propto \right]_{B}^{25} = 58^{\circ}$ (in sthyl acetate,C, 1.2%). Description of the product gave a polysaccharide having the optical activity $\left[\propto \right]_{B}^{25} = 105^{\circ}$ (in water C, 0.8%). This confirmed the homogeneity of the polysaccharide as the original one has the optical activity $\left[\propto \right]_{B}^{25} = 106^{\circ}$

Another portion of polysaccharide was separated by

Zone - electrophoresis in borate buffer (pH 9-3). The

paper chromatogram was cut into 1.0 cm. segments, which

were numbered consecutively from anodic end down to

cathodic end. Each segment was eluted with distilled water,

treated with phenol - sulphuric acid reagent and the

absorbance of characteristic orange-yellow colour was

measured in a Klett-Summerson photoelectric colorimator,

using filter No.50. A plot of the absorbance against segment

number showed only a single sharp peak indicating the

polysaccharide to be homogenous.

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The polysaccharide was slowly soluble in water, $\left[< \right]_{D}^{25} = 106^{\circ} \; (\text{ in water, C, 0.5 g per 100 ml. of solution),} \\ \text{ash content 0.8%. The polysaccharide was found to be free of nitrogen, sulphor and halogens. The methodyl, wronide and acetyl percentage were found to be negligible.$

The complete acid hydrolysis of the polysaccharide with 26-sulphuric acid followed by the paper chromatographic analysis of the hydrolysate revealed the presence of three sugars, D-galactose, D-sylose and L-arabinose. The identity of the sugars was confirmed by their specific optical rotations, preparation of their crystalline derivatives and co-chromatography with authentic samples.

The quantitative estimation of monosaccharide components by periodate oxidation, taking ribose as a reference sugar, showed that galactose, mylose and arabinous are present in the molar ratio 6:7:11 in the polysaccharide.

The graded hydrolysis of the polysaccharide with 0.058 sulphuric acid and subsequent paper chromatographic analysis of the hydrolysate, taken out at various intervals, revealed that galactose wa-s liberated first followed by the liberation of D-styloge and L-arabinose respectively. This shows that most of the xyloge and arabinose are linked together forming the backbone (main chain) of the polysaccharide and galactose units are linked as terminal groups.

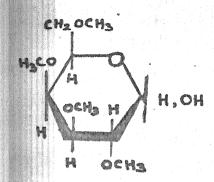
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method using dimethyl sulphate and alkali⁶⁴ followed by purdie's method⁶⁵ with methyl iodide and silver entide. Thus partially methylated product [\propto] 25 = 45° (in chlore-form c-1%), o cm, 33% to ights a fully methylated product, [\propto] 25 =40° (in chloreform, C,1.0%), one, 46.5%. The complete hydrolysis of the methylated polysaccharide and paper chromatographic analysis of the hydrolysate in solvent A, revealed the presence of six methylated sugars. The methylated sugars were separated on a preparative scale by chromatography on whatman No.3 filter paper. The following methylated sugars were identified:

- (I) 2,3,4,6 tetra-0-methyl-D-galactose.
- (II) 2,3,6 tri-O-methyl D-galactose.
- (III) 2,3,4 tri-Compthyl- Daxylose.
- (IV) 2.3 di-Q-mathyl- D-xylose.
- (Y) 2, O-methyl D-Kylose.
- (VI) 2 = 0 = mathyl = L-Arabinose.

Mathylated sugart, had R_{TMG} in solvent A, 0.89,

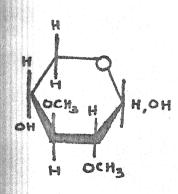


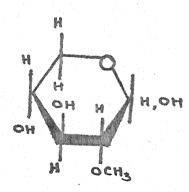
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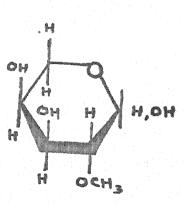
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shows that the methylated sugar IV is, 2,3 -di-O-methyl-D-xylose.

Mathylated sugar V, had R_{200} value in solvant A 0.37, $\left[\times \right]_D^{25} = 25^\circ$ (in water,C,2.3%), m.p. 132 -33°. It formed 2; 0 = mathyl-D-xylose anilide, m.p. 122-24°, $\left[\times \right]_D^{25} + 214^\circ$ (in ethyl-acetate, C, 0.8%). Its discetate, 3-0-mathyl, 3,4 = discetate had m.p. 76-77°, $\left[\times \right]_D^{25} = 39^\circ$ (in chioroform, C,2.8%). Thus the above observations confirmed that the mathylated sugar V is 2-0-mathyl-D-xylose.

Mathylated sugar VI, had B_{200} Value in solvent (A), 0.11, $\left[\times \right]_{20}^{} + 96^{\circ}$ (in water, C, 0.8%) and in literature 15 found $\left[\times \right]_{20}^{} + 100^{\circ}$. It formed 2-G-mathyl-N-phanyl glycosylamine when treated with ethanolic aniline, m.p. 140 - 142° 49a.

The quantitative estimation of methylated sugar, by the method of Hirst and Jones 50, showed that the sugars I, II, III, IV, V and VI were present in the molecular ratio. 2441442:1.

The appearance of 2,3,4,6 -tetra-C-methyl-Dgalactose I, and 2,3,4, tri-C-methyl-D-Mylose III, on hydrolysis of methylated polysaccharide indicates that (two) galactose units and xylose (1 unit) in the polysaccharide occupy terminal position as non-reducing end groups. The presence of 2,3 -di-C-methyl-D-mylose IV, (4 moles) and 2.3.6 -tri-0-mathyl-D-galactose, II. (4 moles) indicates that the backbone of the polysaccharide consists of D-mylose and D-galactose units, through 1 -> 4 linkages, detection of 2-0-mathyl D-mylose, V. (2 moles) shows that mylose units in the main chain per repeating unit of the polysaccharide are linked at position -3 in addition to -1 and -4. A single unit of the 2-0-mathyl-L-arabinose, VI. indicates that L-arabinose is present in the centre of the polysaccharide and linked through, -1. +3 and -4 position.

Determination of terminal groups by periodate cridation and subsequent titration of formic acid liberated corresponds to 0.1476 moles of formic acid per 100 g of the polysaccharids. On the basis of methylation studies, the simplest repeating unit of the polysaccharide, is supposed to consist of 14 sugar moities of which 2 units of galactose and one unit of xylose form terminal groups, considering such a repeating unit, the terminal groups were found 21.69% as determined by periodate oxidation studies, which is in close agreement to that revealed by methylation studies (21.42%).

During the periodate exidation studies the exidised polysaccharide was taken out from the reaction mixture after 60 hours and hydrolysed after destroying the periodate. The paper chromatographic examination of the hydrolysate showed that the presence of xylose was quite prominent.

while no galactors could be detected. The paper chromatography of the hydrolycate of the oxidised polysaccharide
taken out from the reaction mixture after 72 hours should
the presence of archinese. The paper chromatographic analysis
of the hydrolycate of the oxidised polysaccharide taken out
from the reaction mixture after 84 hours should the
shounce of all three sugars. It reveals that galactors
units were completely oxidised within 60 hours, where as
xylose units were exidised within 72 hours and archinese
was exidised after 84 hours. The considerable difference
in the rates of exidation of the component sugars is due to
storic affect resulting from the branched structure of the
polysaccharide. The present imouladge, however, indicates
that this phenomenon is most likely due to cyclic acetal formaction⁵¹.

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The partial acid hydrolysis of the polysaccharide followed by paper chromatographic separation on preparetive scale afforded six oligosaccharides which were detected as follows:

- (1) $3^2 \beta = xy \log 1 xy \log 0$ ($3 \beta = 0$) $xy \log y x an o xy log y x an o$
- (2) Shedymanabiose (C- β -D-sylopyzanosyl-(1->3)-C- β D-sylopyzanose.
- (3) Nichiose (0-/3-Daylophyranosyl-(1-)4)-0-/3Daylopyranose)
- (4) Degalactopyranosyl-(1-)4 L-G-S-D-sylogyranoss).
- (5) 3-0- < -D-cylopyranogyl-1-arabinose

(6) 4-9-x-D- galactopyranosyl -D-galactose.

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Oligosaccharide (1), m.p. 223°, [<721-50° (in water, C. 2.7%), was chromatographically pure in solvent 7 and 3. The molecular weight 420 corresponds to a trisaccharids of pentoses. Acid hydrolysis of the oligosaccharide yielded only mylose. The anomeric configuration of non-reducing xylose units were found to be * B * by ensymatic hydrolysis and negative rotation. Partial acid hydrolysis yielded, xylobiose, zhodymenabiose, corresponding to oligosaccharide (3) and (2) respectively and mylese which were identified by co-chromategraphy with an authentic samples. Periodate oxidation studies showed the consumption of 4.3 moles of meta periodate with the liberation of 2.1 moles of formic acid. Hence the oligosaccharide was identified to be G- β -Docylopyranosyl (1 -> 3)-G- β -Dxyloggranosyl (1-)4 }- IL-xyloggranose i.e. 32- /3 xylosybrylobiose.(Fig. 1)

Oligosaccharide (2), a crystalline sugar, m.p. 191°, $\begin{bmatrix} \mathbb{K} \end{bmatrix}_{0}^{22}$ =21° (in water, C, 2.91 %), was found to be chromatographically pure in two solvent systems F and B. The sugar on acid hydrolysis gave only xylose while the molecular weight of the sugar 296 corresponded to a pentose dissecharide. Shaymic hydrolysis with emulsin showed the presence of β =linkage between the two xylose units. The periodate exidation showed the consumption of 3.26 moles of metaperiodate with the liberation of 1.18 moles of formic acid per mole of the sugar. The oligosaccharide is,

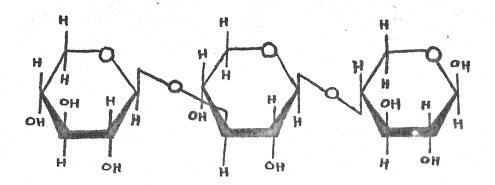


Fig - 1

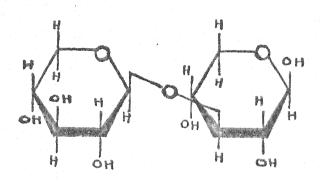


Fig - 2

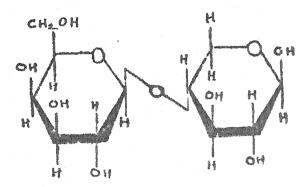
Fig-3

therefore, identified to be ghodymenabless ($G_{-}\beta$ -D_
mylopyranesyl = (1->3) 0 = β - D-mylopyranese (Fig-2).

The identity was confirmed by co-chromatography with an authentic s-amples

Oligosaccharide (3), m.p. $187-88^{\circ}$, $[<]_{D}^{20}-26.4^{\circ}$ (in water, C, 3.5%), was chromatographically pure in solvent systems F and 8. Acid hydrolysis showed the presence of xylose only. The molecular weight of the sugar was 296, corresponded to a disaccharide of xylose units. The periodate oxidation showed the liberation of 2.21 moles of formic acid with the consumption of 4.31 moles of metaperiodate per mole of the oligosaccharide. Hence the oligosaccharide was assigned the structure $(-)^3$ -Daxylopyranosyl $-(1-)^4$ - xylopyranose. (Fig-3). The identity was further confirmed by co-chromatography with an authentic sample.

Oligosaccharida (4), m.p. 190-92°. [5] 15° (in water), was shown to be chromatographically pure in solvent I. On acid hydrolysis revealed the presence of galactose and xylose units. The quantitative estimation by the method of Hirst and Jones 13 showed the molar ratio 1:1 between the two sugars in the oligosaccharide. The molecular weight 296, showed it, to be a disaccharide. Periodate oridation studies afforded the liberation of 2.12 moles of formic acid and consumption of 4.14 moles of periodate per mole of the eligosaccharide. (Fig.4). Thus the sugar was confirmed to be D-galactopyranosyl -(1-)4)-



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Fig-4

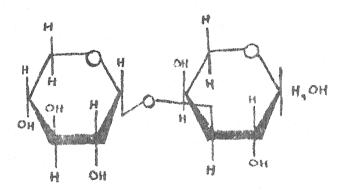


Fig-5

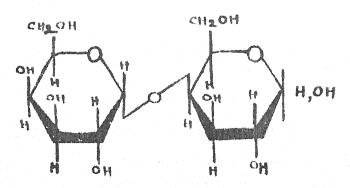


Fig-6

o Daylopyranose.

Oligosaccharide (5). syrup [x] 25 + 172-)180° (in water) R, value in solvent R was found 0.48 chromatographically pure in solvent R. The complete acid hydrolysis followed by paper chromatographic analysis revealed the presence of two sugar & Daxylose and Larabinose. The quantitative estimation by the method of Hirst and Jones 43 showed the molar ratio to be 1:1 between the two sugars in the oligosaccharide. Periodate oxidation studies showed the consumption of 3.25 moles of periodate with the liberation of 1.2 moles of formic acid. Methylation study of the oligosaccharide followed by acid hydrolysis of the fully methylated derivative afforded 2,3,4 -tri-0-mathyl Daylose and 2-0-methyl -L-arabinose in equal proportions. The oligosaccharide was not hydrolysed with emulsin indicating the <-linkage between the two sugars. The results proved that oligosaccharide was 3-0- K-D-Mylopyranosyl-L-arabinoss.

Oligosaccharide (6), was also a syrup, \square_D^{25} +175° (in water C,1.2 %). The digosaccharide was shown to be chromatographically pure in solvent-G. On acid hydrolysis followed by paper chromatographic analysis showed the presence D-galactose only. The molecular weight 347 calculated for C_{12} H_{22} O_{11} 342 corresponded to a hadose disaccharide. Periodate oxidation studies showed the consumption of 4.22 moles of metaperiodate and liberation of 2.18 moles of formic acid. Nothylation study of the oligosaccharide followed by acid hydrolysis it afforded

2,3,4,6-tetra-C-methyl-D-galactose and 2,3,6 -tri-C-methyl-D-galactose which was transformed by bromine water cridation in alkaline solution afforded formaldehyde 64.

It was concluded that the biose linkage 65 was of 1->4 type.

Oligosaccharide was not hydrolysed with emulsin it follows that the disaccharide must be 4-C- (-D-galactose.(Fig. 6).

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On the basis of the results obtained so far particularly from methylation studies, graded and partial hydrolysis, following valuable information could be derived:

- (i) The main chain of the polysaccharide consists of β =(1=>4) linkage and β =(1=>3) linkage of xylose-galactose units alongwith β =(1=>4) linkage of linkage of galactos and arabinose units.
- (ii) Two units of galactose are linked as terminal groups in the main chain through β (1->4) linkages.
- (iii) One mylose unit per repeating unit of the polysaccharide is linked through \leftarrow (1 \Rightarrow 3) linkage in the main chain as terminal group.
- (iv) Only one unit of arabinose is linked through $\beta = (3 \Rightarrow 4)$ linkage in the centre of the polysaccharide.
- (v) From the above information, it is also clear that the galactose units in the side chain are limbed at the same xylose units in the main chain

which linked through β (1-3) linkage in the main chain.

Taking all the experimental evidences into consideration together with the structure of different oligosecharide, the following most probable structure has been assigned to the polysaccharide from the seeds of Zizyphus rugosas

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$$A = \beta = 1$$

Cal.p = D-galactepyranose

Arab p = I Arab inopyranose

MAD = D-Mylobaranose

The structure contains 14 units of monosecharide per repeating unit which fully explains the formation of oligosecharide as obtained by partial hydrolysis and agrees well with the analytical data of the polysecharide. The dotted and doubly agrowed dotted lines shows the probable

mode of fission of the linkages ouring the partial acid hydrolysis. The arrowed dotted lines indicated secondary hydrolysis.

The polysaccharide described above should consume 14 moles of metaperiodate with the liberation of 3 moles of formic acid per repeating unit of 14 sugar units. The actual consumption of periodate 14.08 and the biberation of formic acid 2.993 moles have been determined for per repeating unit of the polysaccharide, which are in close agreement to the calculated values.

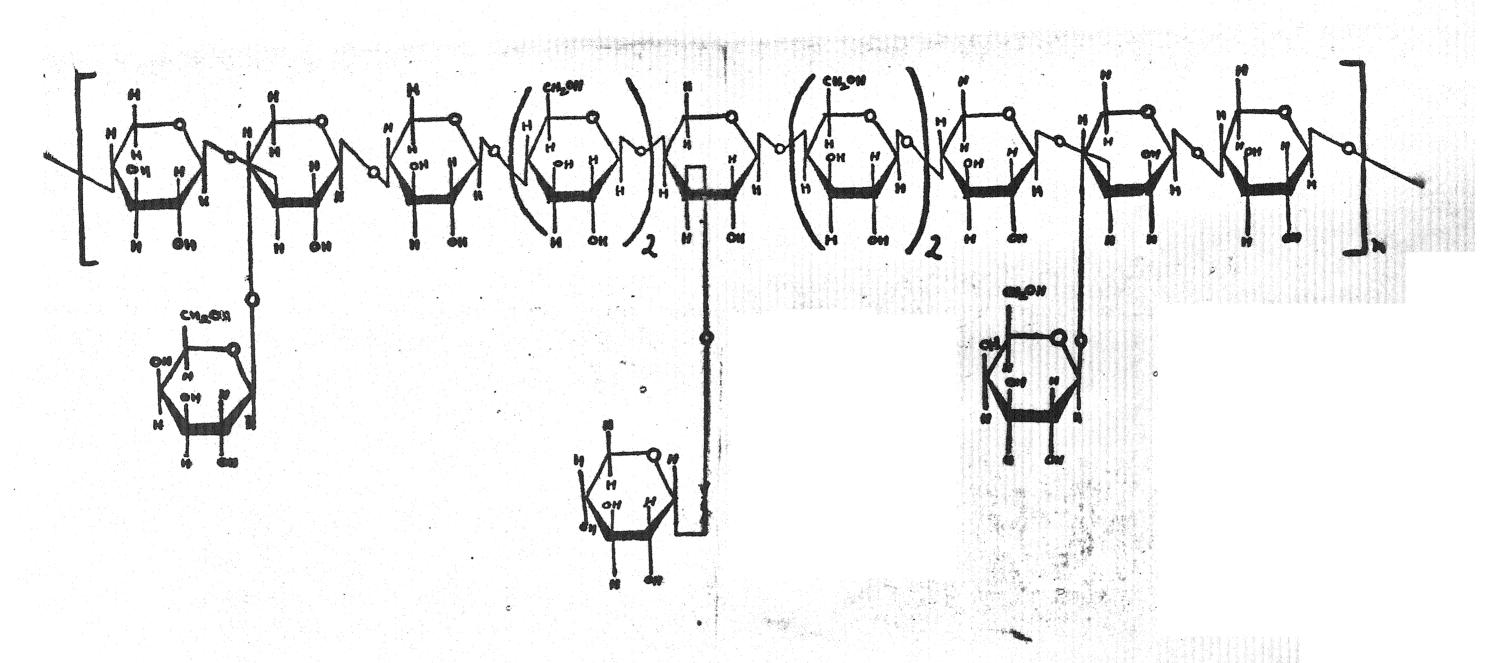
Similar other structures may be possible but they are less probable because the formation of eligosaccharides as obtained in the present case might not be possible.

II. 4 EXPERIMENTAL

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All evaporation were carried out under reduced pressure at low temperature unless openified otherwise. Residues were dried in vacuum at room temperature over anhydrous calcium choride. All specific rotations are inequilibrium values and all melting points are uncorrected. Paper chromatography was performed at room temperature by descending technique on Whatman No.1 filter paper unless stated otherwise, using following solvent system:

- (A) n-Butanol ethanol water $(4:1:5)^{50}$
- (8) n-Sutanol acetic acid-water (4:1:5)53
- (C) n-Sutanol-isopropanol water $(11:6:3)^{54}$



STRUCTURE OF THE NEW POLYSACCHARIDE FROM THE SEEDS OF ZIZYPHUS RUGOSA

(D)	Bensene - ethanol - water	(169:47:15)55
(3)	Butanone - Water	(11: 1)56
	Sthylacetate -pyridine - water	(11:4:3)57
. *	Sthylacetate -pyridine - water	(2:1:2)58
	n-Butanol - ethanol - water	(40:10:19)59
-	n-patanol - ethanol - water	(5:1:4)60
	Pyridine -ethylacetate - water	(1:2.5:3.5)61

The spots were located by spraying a chromatogram with aniline hydrogen phthalate⁶² and heating it at 110-120⁶ for 10 - 15 minutes. Spectrophotometic determinations were carried out by a modification. of phenol - sulphuric acid method⁶³. Klett-Summerson photoelectric colorimeter was used for measuring the absorbance.

IT. S ISOLATION OF THE POLISACCHARIDE

The dried and crushed seeds (2.0 Mg) were extracted successively with petroleum ether (60-80°) and ethanel. The extracted seeds were dried and then suspended in distilled water (2 litre) containing 1% acetic acid. The mixture was stirred mechanically for 6-10 hours to extract the mucilage as much as possible and squeezed out through a muslin cloth. The process was repeated six times when practically no precipitate was obtained by adding the extract to an excess of ethanol. The combined extracts were filtered thrice through a thick cottem pad, placed over a cloth in a Buchner funnel to remove the suspended fine particles. The clear mucilage solution so obtained was added slowly to a large excess of ethanol with constant vigorous stirring

when a fibrous colourless precipitate of the crude polysaccharide was obtained. It was filtered, washed with ethanol, followed by absolute ethanol and dried in vacuum at room temperature (4.5 gm; ash 5.3%).

II.6 PURIFICATION .

The dried crude polysaccharide was dissolved in distilled water (2 litres) containing 1% acetic acid with constant stirring. The solution was filtered and added very slowly to ethanol (8 litres) with constant and vigorous stirring and kept overnight. The precipitated polysaccharide was filtered and the above process was repeated thrice, to get a white fibrous mucilage (39 g s ash 0.5 %).

II.7 HOMOGENEITY OF THE POLICIACCHARIDS

The homogeneity of the polysaccharide was checked by the following methods :

II.7.1 (a) Fractional precipitation

The pure mucilage (5 g) was dissolved in distilled water (500 ml). It was then added slowly to ethenol (500 ml) and the precipitated polysaccharide (Fraction I) was filtered, washed with ethanol followed by absolute ethanol and dried in vacuum. The filtrate was treated with another 1000 ml of ethanol with stirring and precipitated polysaccharide (Fraction II) was filtered, washed with ethanol and dried in vacuum. Both the fractions alongwith the original polysaccharide were hydrolysed separately with 26

sulphuric acid. The sugar present in each hydrolysate were first identified by paper chromatography with authentic sugars using solvent (C) and then separated on two sheets of thatman No.1 filter paper using the same solvent. The sugars were eluted with water and estimated quantitatively by periodate oxidation method⁴³. The sugars eluted from one sheet were estimated by titration of formic acid liberated with standard alkali solution whardas the sugars from the other sheet were estimated by the method of consumption of periodate. The ratio of D-galactose, D-xylose and L-arabinose in both fraction was found almost the same (6:7:1), indicating the purified polysaccharide to be homogeneous.

Tt.7.2 (b) scetylation and Descetylation

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The pure polysaccharide (3 g) was mixed thoroughly with anhydrous sedium acetate (10 g) and mixture was suspended in acetic anhydride (30 mb). After refluxing over a water-bath for 16 hours, the mixture was cooled to room temperature, and poured over crushed ice with constant stirring and then left overnight. The grayish white precipitate was filtered, washed with water and dried in vacuum. The dried mass was then dissolved in minimum quantity of acetone and the solution was poured slowly in distilled water, where upone a fine fibrous precipitate was obtained. This precipitate was filtered, washed and dried in vacuum 2.1 g. [] 55° 58° (in ethyl acetate, C, 1.2%).

The dried acetylated polysaccharide (1.8 g) was dissolved in acetone (32 ml) and 50% potassium hydraxide

The original polysaccharide $\left[\prec \right]_D^{25}$ -106.5° (in water, c.o.5%) and the polysaccharide obtained after descetylation had almost the identical specific rotation indicating the homogeneity of the polysaccharide.

II.7.3 (c) Zone - Electrophoresis

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A strip support (15 cms x 45 cms) of Whatman No.1 filter paper was marked with a pencil in middle to indicate the starting line. 0.5% solution of polysaccharide (50 ml) was placed on starting line as a compact band. After drying at room temperature the strip was sprayed with borate buffer (pH 9.3) and suspended horizontally in the electrophoresis tank containing two electrode compartments each having approximately 400 al of borate buffer (pH 9.3). After electrophoresis at 260 V and 12.5 mA for 6.5 hours, the paper strip was dried. It was then cut lengthwise into 1 cm sagmants, which were numbered to the cathode end. The material from each numbered strip was eluted with water (6 ml) and filtered through glass wool. The filtrate (5 ml) was placed in a hard glass boiling tube with 8.5% aqueous phenol (1 ml). To the tube, concentrated sulphuric acid (15 ml) was added rapidly. The tubes were allowed to cool at

room temperature. The absorbance of characteristic yellow orange colour was measured in a Klett-Ammerson photoelectric colourimeter using filter No.50. A blank was also run under the same conditions but without polysaccharide.

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The reading so obtained were plotted against the segment number counted from the anode end to the cathod end.

Only one sharp peak was obtained indicating the polysaccharide to be homogenous.

TABLE - I

	A. A. Section of the second section of the section of the second section of the section of			
Secont No.	Klett reading of elute	Blank Klett reading	Corrected Klutt reading	Absorbance
	27		2.0	0.004
2	24	23	1.0	0,002
	24	3	1.0	0.002
4	20	25	3.0	0.006
5		25	3.0	0.006
6	23	2.1	3.0	0,004
7	23	21	2.0	0.004
	29	25	4.0	0,008
		23	2.0	0.006
10		25	3.0	0.006
11	24	21	3.0	0.006
12		23	2.0	0.004
13	45	25	7.0	0.014
34	40	28	12.0	0.024
	47	20	27.0	0.054
36	30	25	23.0	0.026
2.7	27	24	8.0	0.016
18	20	24	2.0	0.004
19	23	22	1.0	0.003
20	23	21	2.0	0.004
31	23	22	2.0	0.004
22	25	21	4.0	0,008
23	24	21	3.0	0.006
24	21	25	2.0	0-004
25	20	27	1.0	0.003
36	28 27	3	2.0	0.004
28		25	1.0	0.006
29			2.0	0.004
30	46	3	2.0	0.008

Absorbance was measured on 5 ml portion of coloured solution.

Absorbance = 2 x Klett reading .

ILS BHCWEAR

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The Gried polysaccharide (0.3g) was ignited in a silica crucible previously heated to a constant weight.

After ignition the crucible was cooled in a desiccator and weighted. From the weight of residue (0.0010 g), the ash content was calculated to be 0.8%.

IT.9 PHYSICAL AND CHEMICAL EXAMENATION

It was a fibrous white powdered, very light in weight, slowly soluble in wa-ter, [K] = 106.5° (in water, C, 0.5 g per 100 ml of solution). For the purpose of optical rotation, the solution was filtered through a sintered funnel to get a clear solution and the amount of polysecharide in the solution was determined colorimetrically. The polysecharide was found to be free of nitrogen, sulpher and halogens. It did not reduce Penling's solution.

II.10 EXAMINATION OF PRISE SUGARS

The polysaccharide was examined for free sugars by applying three apots of its solution in water on a strip of Whatman No.1 filter paper (15 cms x 45 cms). The paper was developed in solvent (A) for 36 hours, dried and cut lengthwise into three strips, each containing one spot. The three strips were sprayed with three different reagents using maphthoresorcinol and trichloroscetic seid (gives colour with ketoses only) on one, aniline hydrogen

phthalate on the second and silver nitrate in acetone followed by ethanolic sodium hydroxide on the third. The first two paper dried in the over at 120° and the third was air-dried. Home of the strip showed any spots hence the polysaccharide did not contain any free sugar.

II.11 METHORY, GROUP DETERMINATION

The percentage of mathomyl groups was determined by the mathod of Balcher, Fildes and Butten 68 and was found to be 0.31%.

II. 12 ACSTYL GROUPS DETENBENATION

The method by Balcher and Godbert 49 was followed for the determination of acetyl group percentage with and without mucilage found scetyl 0.90%.

II. 13 URCHOID CONTENTS DETERMINATION

The uronoid contents were found to be negligible by the semi-micro ma-thod of Barker, Foster, Siddigul and Stacey 70

IL. 14 HADROLYS IS OF POLYSACCHARDER AND DETERMINATION OF MONOSACCHARDS

The purified mucilage (1.5 g) was dissolved in 2N sulphuric acid (100 ml) and was hydrolysed on a water-bath for about 24 hours. The hydrolysate was neutralized with barium-carbonate, filtered and concentrated under reduced pressure. The hydrolysate was examined for monosaccharide as described below:

II.14 (a) Paper chromatography

The spots of the hydrolysate were applied on two sheets of whatmen No.1 filter paper. The papers were developed separately in solvents (A) and (B) by descending unidimensional technique. The chromatograms were air-dried and sprayed with aniline hydrogen phthalate. On heating themin an oven at 120°, each chromatogram showed three spots. The R_g and R_g values of the three spots corresponded to Degalactose. Decylose and Learabinose as given in the following table.

Table - 2

	Solvent(A)			Solvent (8)	
ident if led	A.	R _G 71	R.	R ₂ 53	
	found	given	found	givan	
D-galactosa	0.06	0.07	0.16	0.16	
Casyloge	0.16	0.15	0.29	0.28	
l-/cabinose	0.11	0.12	0.20	0.21	

G = 2,3,4, 6-Tetra-O-methyl-D-glucose.

The identity of the three sugars was further confirmed by co-chromatography with an authentic samples of the sugars.

II. 14 (b) Column Chromatograchy

A portion of hydrolysate was dissolved in a small amount of equeous methanol (1:1) and absorbed over a well washed column of cellulose(1" x 15"). The column was left overnight and the separation was effected with solvent (A) and several fractions (15 ml) each were coellected. Each

fraction was analysed by paper chromatography with authentic samples of D-galactose, D-xylose and L-arabinose in solvent (B). The fraction 1-10 containing same sugar ware combined together and concentrated to give D-xylose. It was recrystallised from aqueous methanol, [x] 30+17.7° (in water, C, 1.15%). The melting point of the sugar was found to be 142-44°. The following derivative was prepared.

D-Wylose Phenyl Geasone Derivative

The osamone of the sugar was prepared by heating (250 mg) of sugar, 50 mg of phenyl hydramine hydrochloride and 0.3 g of sodium scetate dissolved in 5 ml of water in a test tube and heated for 30 minutes on a boiling water-bath. Precipitate of the osamone started appearing after 7 minutes. The flocculent precipitate was separated with water, recrystallised from 50% ethanol, m.p. 161° resembling to an authentic sample.

The fraction 15-30 containing same sugar were mixed and concentrated to give D-galactose. It was recrystallised from aqueous methanol, $[\propto]_D^{25}$ 478,5° (in water.C. 0.6%). The melting point of the sugar was found to be 167°. The following derivatives were prepared.

(1) D-Galactosa Phenyl Hydrazone

Pound

Given (Lit.)72

m.p. 152-53⁰

154-55°

(11) M-p-Nikrophenyl-D-Galactosylamine

In a microtest-tube galactose (25 mg), p-nitroaniline (25 mg), a drop of glacial acetic acid and 2 drops of methanols water (8;1 v/v) were taken. The whole mixture was boiled for

8 minutes and kept overnight in a refrigerator. The exystalline product was filtered, washed with cold ethanol, ether and dried in vacuum. It melted at 217-18° after recrystallisation from methanol. Lit. 73 m.p. 219°.

I-arabinose phenyl osazone derivative

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The sugar (0.2g) gave phenyl esasons on heating with phenyl hydraxine hydrochloride (0.4 g), exystalline sodium acetate (0.6 g) and water (6 ml) on a boiling water-bath for 30 minutes. The solution was cooled and the precipitate phenyl esasons was filtered and recrystallised from aqueous ethanol, map, and mamap, with an authentic sample 163-64° 75.

II.14 (c) Thin-layer Chromatography

The plates were prepared from alurry of silic gel-G in O.1N solution of boric acid and the spots of hydrolysate alongwith bensene : acetic acid : methanol (1:1:3)⁷⁶ and airdried. These plates were sprayed with aniline hydrogen phthalate paagent. On heating them at 120° in an oven, three spots corresponded to D-galactore, D-cylose and L-arabinose were observed.

II.15 QUANTITATIVE SETIMATION OF MONOS ACCHARIDE

The method due to Hirst and Jones 43 was applied for quantitative estimation of component sugars of the

polysaccharide.

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The polysaccharide (300 mg) was dissolved in 21 sulphuric acid (20 ml) in a 250 ml round bottom flash. The flash was then heated for 24 hours on a water-bath. After cooling to room temperature the hydrolysate was diluted to 30 ml and them D-ribose (20 mg) was added to it. The whole solution was shaken well and transferred to the beaker. The solution was neutralised with barium carbonate and filtered. The filtrate and the washing of the barium carbonate were concentrated and then made upto 10 ml.

Six sheets (30x45 ems) of Whatman No.1 filter paper were used as paper chrematography. Three guide strips(4x45cms) two on either edges and one in centre, were marked on each paper. A portion of above solution was placed along the starting line (8 cms away from the upper edge) of the three sheets, whereas the remaining three sheets were used as blanks. A guide spot was placed in the centre of each guide strips. All the sheets were developed in solvent (C) for 48 hours. After drying the chromatograms, guide strips were cut lengthwise, sprayed with aniline hydrogen phthalate and heated in an oven at 120° to locate the position of sugars. With the help of guide strips, appropriate sections of unsprayed portion were eut alongwith the blank strips of same dimension from the blank chromatograms. Each section (with and without sugar) was cut into small pieces and extracted separately with 10 ml of hot water. The eluted sugars were then oxidised with 0.25M sodium metaperiodate(5ml). The liberated formic acid was titrated with standard alkali, after destroying the excess of metaperiodate with sthylene glycol (2 ml), using methyl god as

indicator. Slank readings were substracted to get the titre values.

	Value usos	s of alkalis		corresponding asso		
		2	G	A		6
Calactosa	9.30	7.60	10.52	2.756	2.300	3.104
Xy 1000	10.48	8.74	11.90	3-221	2,486	3.627
Arab inose	1.50	1.24	1,68	0,461	0.381	0.516

* Strength of NaOH = 123

3.7464

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Assuming complete recovery of D-ribose, the above results indicate that in the polysaccharide D-galactose, D-mylose and L-Arabinose are in the molar ratio of 6:7:1.

II. 16 GRADED HEDROLES IS 77 OF THE POLIS ACCHARIDE

The polysaccharide (200 mg) was dissolved well in 0.05N sulphuric acid (20 ml) and the hydrolysis was carried out over a boiling water-bath. The hydrolysates were taken out at various intervals, and examined chromatographically without removal of sulphuric acid using solvent (B) for the purpose of irrigation of the paper. Results are given in table -4.

Table - 4

(in minutes)	Sugar ident if led	No. of other apots
	Galactose (Faint)	
10	Galactose (Faint)	
25	Galactose + xylose(Faint)	
20	Same as above	
30	Same as above	
60	Same as above	Dro spots
90	Same as above	Two spots
120	Galactose + xylose + Arabinose(Very Faint)	Three spots
180	Galactosa + xylose + Akabinose	Pour apots
340	Same as above	Same as above
420	Same as above	Same as above

During graded hydrolysis of the polysaccharide galactose was found to be liberated first followed by xylose and them arabinose. The earliest release of D-galactose and simultaneously of D-xylose and i-arabinose (Faint) leads to the consumption of D-galactose are present as terminal groups and some units of D-xylose are also present as terminal groups instead of main chain of the polysaccharide. As galactose is liberated earlier than xylose, this is most probably attached to the main chain by more easily hydrolysable limitage. Arabinose is liberated after 120 minutes indicating that I-arabinose is present as main chain of the polysaccharide and this is most probably attached in the main chain by hardly hydrolysable limitage.

II.17 METHYLATION OF POLYSACCHARIDS

The polysaccharide was mathylated first by the mathod of Parilds, ingle and Maide 44 gollowed by Purdie's mathod 45.

The polysaccharide (6.8 g) was dissolved in minimum amount of water and them taken in a conical flask fitted with 24 joint. Dimethyl sulphate (40 ml) and 40 % sodium hydroxide (80 ml) were added dropwise with constant stirring by magnetic stirrer. The temperature was maintained between 40-50°. After repeatition of the above procedure, the solution was concentrated under reduced pressure and filtered to remove the modium mulphate. The filtrate was again concentrated to a thight symp and dissolved in acetone. This was then methylated by repeating the above procedure thrice. The finally concentrated solution was entracted thoroughly with chloroform. The extracts were dried over anhydrous sodium sulphate and the solvent distilled off under reduced pressure. The partly methylated product was brownish mass. (5.9 g), -OCH3, 33%, [4] 25 -45° (in chloroform, C, 1 per 100 ml of solution.

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The partly methylated polyeaccharide was further methylated by Purdie's method⁴⁵. The partly methylated polyeaccharide (5.6 g) was dissolved in mathanol (36 ml) in a comical flask fitted with three necked multiple adapter. The temperature was maintained at 40.50° by placing the comical flask, fitted with air-condenser having fused CaCl₂-tubes in a through containing water over the magnetic stirrer. Methyl iodide (9 g) and silver exide (6 g) were added with continuous stirring in several equal instalments, each after half an hour interval. After the final addition the reaction mixture was heated for four hours on a water-bath under reflex and then filtered after cooling the contents. The silver salts were exhaustively extracted with chloroform

under reflux. The combined filtrate and extracts were evaporated under reduced pressure and the resulting syrup was remethylated thrice under the same conditions. The fully methylated polysaccharide was obtained as a deep brown coloured product. (4.8 g) = OCH₃, 46.5 %, $[K]_D^{25} = 40^{\circ}$ (in chloroform, C. 1.0%).

DESTURED OF METHYLATED SUGARS

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The hydrolysis of the methylated polysaccharide was carried by slight modification of method due to Bouveng etcal 78 The methylated polysaccharide (100 mg) was dissolved in 85% formic acid (20 ml) and solution was refluxed for 4 hours on a water-bath. The solution was than cooled and concentrated under reduced pressure and traces of formic acid were removed under vacuum. It was dissolved in 0.25N sulphuric acid(10 ml) and the hydrolysis was carried out for 16 hours on a water-bath. The hydrolysate was cooled, neutralised with barium carbonate and filtered. The residue was washed with water followed by ethanol. The combined solutions were concentrated under reduced pressure to light brown syrup. The methylated sugars were separated on Whatman No.1 filter paper using solvent (A). The chromatograms showed six spots after spraying with aniline hydrogen phthalate and drying at 120°. The $R_{\rm T/4G}$ (TMG = 2,3,4,6-Tetga-O-methyle -D-glucose) value of each methylated sugars was calculated in solvent(A). These values were compared with that given in literature as shown in the following table.

2ABLS = 5

	Solvent(A)		
Methylated sugars identified	R _{TMO} found	gavea	
2,3,4,6-Tetra-G-methyl-D-galactos	0.89	0.88	
2,3,6-Tri-Gemethyl-Degalactose	0.70	0.71	
2,3,4-tri-G-mathyl-D-cylose	0.95	0.94	
2,3,-di-Genethyl-Daylose	0.75	0.74	
2.0 -makhyl-Dastylose	0.37	0.38	
2, G-mathyl-L-arabinose	0.11	0.12	

II.19 QUANTITATIVE ESTURTION OF METHELATED SUGARS

as described above. After hydrolysis, glucose(60 mg) was added to hydrolysate. It was then neutralised with parium carbonate and filtered. The residue was washed with ethanol. The filtrate and washing were concentrated under reduced pressure to a syrup. A portion of the syrup was dissolved in acetone and applied on three sheets (A,B, and C) of Whatman No.1 filter paper. Such having three guide strips. The papers were irrigated with solvent(D) alongwith three blank sheets. After development of chromatograms and locating the sugars on guide strips, appropriate sections, containing sugars were cut from the unsprayed portion of the chromatograms. The sugars were cluted with 10 ml of water.

The methylated sugars were estimated by alkaline hypotodite method⁵⁰. The eluted portions were taken in 50ml conical flasks separately provided with ground glass joint stopers, and a solution (2 ml) containing 0.2M sodium bi-carbonate and 0.2M sodium carbonate was added solution of

and the flask was stoppered. The experiments as corresponding blank elutes were also carried out in the same way. After three hours, the reaction mixture was acidified cautiously with 2M sulphuric acid and 15% potageium iodide solution (2ml) was then added to it. The liberated iodine was titrated against 0.01M sodium thiosulphate solution using starch as indicator. The results are given in table-6.

TABLE - 0

Fraction & sugar		ar Volume of 0.01N Corresponding amo					
		λ		G	Α	13	C.
A	2.3.4.6-tetra-0- mathyl-D-galactose	2.02	2.06	2.40	2.201	2.245	2.616
B	2,3,6-tri-O-methyl- D-galactose	4.32	4.40	5.16	4.406	4.496	5.218
C	2,3,4-tri-Casthyl- Daylose	1.26	1.30	1.50	1.096	1.131	1.305
D	2.3-di-G-methyl-D- xylose	5.52	5.62	6.52	4.416	4.496	5.216
8	2-C-methyl-D-cylose	3.02	3.08	3.60	2.204	2.248	2.620
P	2-0-mathyl-1- arabinose	1.50	1.54	1.80	1.095	1.124	1.314
G	D_Glucose	3.42	3.50	4.08	3.078	3.150	3.672

The above results correspond to an average molar ratio between AtB1C3D1E3F as 2:4:1:4:2:1. The methylated augars were calculated as the methyl ethers of anhydrohescose and anhydro-pentose i.e. $C_6H_{13}O_5$, $C_7H_{14}O_5$ and $C_8H_{16}O_5$ for mono-, di-, and tri- 0-methyl-D-Mylose respectively and $C_9H_{16}O_6$ and $C_{10}H_{20}O_6$ for tri-, and Tetra-0-methyl-D-galactose respectively and $C_9H_{18}O_6$ and $C_1O_{20}O_6$ for mono-0-methyl-L-arabinose. An average recovery of the methylated polysaccharide was found

to be 90.90% assuming 100% recovery of D-glucose.

II. 20 CHARACTERESATION OF METHYLATED SUGARS

to the method of Garage and Lindbarg. Mathylated polysecharide (4.0g) was dissolved in 72% sulphuric acid (50 ml).
The solution was kept for one hour at room temperature(25°) and then diluted to 200 ml. Further hydrolysis was carried out by heating for 4 hours on a water-bath. The solution was cooled neutralised with barium carbonate and filtered. The residue was washed with water followed by ethanol. The solutions were concentrated to a syrup under reduced pressure.

The mixture, containing different mathylated sugars, was resolved into six fractions on whatman No.3 filter paper using solvent (D). Strips, containing different individual methylated sugars, were eluted with water. The clutes were concentrated separately under reduced pressure and marked as fractions I, II, III, IV, V and VI.

IX. 20.5. Fraction I

A solid, $R_{\rm PMS}$ in solvent (A) 0.89, found ONe, 51.5% calculated for tetramethyl hexose. ONe 52.4%, $[<]^{25} + 123^{\circ}$. (inwater, C.0.5%). Lit 86 ,87.88 for 2.3.4, 6-tetra-0-methyl-D-galactose $[<]^{16}_{\rm D} + 142^{\circ} \Rightarrow + 117^{\circ}$ (equil.)(in water C.1.1%), m.p. 71-73°. It gave a red colour with p—anisidine hydrochloride spray in Antanol and a brownish red colour with aniline hydrogen phthalate. On treatment with ethanolic aniline gave 2.3.4.6 -tetra-0-methyl-N-phenyl-D-galactosylamine, map: 189-60°, $[<]^{25}_{\rm D} + 80^{\circ}$ (in acetono.C. 1.1%) Lit maps 193-94°

Lit so m.p. 192° [N] p - 77°. Therefore, the identity of methylated sugar is established as 2,3,4,6-tetra-C-methyl-D-galactose.

11.20.2 Fraction II

Solid, map. 96°, $R_{\rm DMG}$ in solvent (A) 0.70, $[\le]_{\rm D}^{25}$ -40 \longrightarrow - 36° in water, Lit. 47 , map. 96°, $R_{\rm DMG}$ in solvent (A) 0.70 in Lit, $[\le]_{\rm D}^{\rm c}$ - 44 \Longrightarrow -37°. It formed 2,3,6-tri-G-methyl amide with the treatment of concentrated amounts solution, map. 134°. Lit. 46 , map. 135°. It formed 2,3,6-tri-G-methyl phenyl hydranide with phenyl hydranide hydrochloride having map.173° Lit. 48 , 175°.

IL.20.3 Fraction III

Syrup, it could not be recrystallised. The $R_{\rm TMG}$ in solvent (A) 0.95, optical rotation of sugar was found to be $[\mbox{$$

The anilida of the sugar was prepared by reflexing the dry syrup (38 mg) with freshly distiled dry aniline (120 mg) for three hours in a water-bath (85-95°) in absolute ethanolic solution (5 ml). Ethanol was distilled off and the whole viscous mass was kept in the refrigerator for seven days. The 2.3.4-tri-G-mathyl-D-xylophranosyl anilide failed to crystallise. It came out as a white powder by the addition of 3-4 drops of dry acetone. The precipitate was filtered out and dried, (yield 10 mg). The m.p. of powder was found to be 94-95°, $[< J_{\rm p}^{22} - 83^{\circ}]$ in ethanol.C.2.5%) Lit ⁶³, m.p. 129° [> 54 > 47° and 12°, m.p. 91°.

The methodyl value of the derived entitle was found to be 33.5% ($C_{14}^{H}_{21}^{O}_{4}^{O}$ N requires—OMe, 34.8%).

The sugar in this fraction was thus identified as 2,3,4-tri-C-mathyl-D-xylose.

11.20.4 Fraction IV

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Syrup, $R_{\rm TMG}$ in solvent (A) 0.75, found ONe, 34.8%, dimethyl xylose, $C_{\gamma}H_{14}O_{5}$, requires—OCH₃, 34.8%. The optical rotation of the sugar $\left[\propto\right]^{20}$ + 22.5 (in water, C.4.6%). Lik ⁸³. $\left[\swarrow\right]^{15}$ + 23°.

The anilide of the sugar prepared by the method of Hampton 84. The dry syrup (200 mg) were refluxed for six hours with 1.5 ml of freshly prepared distilled dry aniline dissolved in 10 ml of absolute ethanol. The ethanol was distilled eff and the bulk of the aniline was removed under high vacuum, (5-6 mm of mercury) at 65-70° (bath temperature). The syrup mass was kept in the refrigerator for 72 hours, when tiny exystals (plates) were observed. The adhering aniline was removed by the addition of dry ether, and the crude light brown crystals was filtered out, washed with ether, and dried. (yield 38 mg), m.p. 137-38° for 2.3, di-0-methyl-Damylopyramosyl anilide in Lit 83. m.p. is 145° and optical rotation [4] 192° (in ethyl acetate C.0.3%) and in Lit 85 [4] +185° (in ethyl acetate).

The methoxyl content of 2,3-di-0-methyl-D-xylopyranose emilide (recrystallised was found to be 25.2% calculated for $^{\rm C}_{13}{}^{\rm H}_{19}{}^{\rm O}_4{}^{\rm N}$, cms, 24.8%). The sugar present in this fraction was identified as 2,3-di-0-methyl-D-xyloss.

11.20.5 Fraction V

calculated for mone methyl pentose, $C_8H_{12}O_8$, one, 18.96%. m.p. 132-33°, $\left[\times \right]^{25}$. 25° (in water C.2.3%). Lit anp. 135-37°, $\left[\times \right]^{25}$. \times 25° (in water). Lit anp. 132-33°. $\left[\times \right]^{25}$. \times 26° (in water). It formed 2. C. methyl. L. xylose anilide on treatment with ethonolic aniline, m.p. 132-24°, $\left[\times \right]^{25}$. \times 214° (in ethyl acetate, C. 0.8%). Lit a. m.p. 125-26°, $\left[\times \right]^{25}$. \times 214° (in ethyl acetate).

on acetylation of sugar with anhydrous sodium acetate and acetic anhydride a grayish white precipitate was obtained. The dried mass dissolved in minimum quantity of acetone and the solution was poured slowly in distilled water, where upon a white crystalline compound 3-0-methyl-Daxylose, 3.4-diacetate, m.p. 76-77°, [X]²⁵ = 3.9° (in chloroform, C,2.8%). Lit 82 m.p. 78-79°, [X] = 38° (in chloroform).

II.20.6 Fraction VI

syrup, R_{TMS} in solvent (A) 0.11 in Lit⁴⁹. 0.12. . [K]_D + 96° (in water, C, 0.8%). Lit.^{48,49} [K]_D + 100° (in water). It formed 2-0-methyl-N-phenyl glycosylamine when treated with ethanolic aniline having m.p. 140-42° in Lit.⁴⁹(a) m.p. 142°.

II.21 PERICUATE CKIDATION OF THE POINSACCHARDS

II.21(a) Liberation of formic acid and estimation of end group

The polysaccharide (500 mg) was dissolved in water

(5 ml) and in this solution, potassium chloride(0.5 g) and 0.25-M sodium metaperiodate (60 ml) wore added. The volume was made upto 140 ml with water. In a blank experiments, potassium chloride (0.5 g) and (0.25 M) sodium metaperiodate (60 ml) were diluted to 140 ml with distilled water. The emidation was carried out in a dark at room temperature, 5 ml ef alique were drawn at various intervals alongwith blank and excess of metaperiodate was reduced with 2 ml of ethylene glycol. The liberated formic acid was titrated against N/110 sodium hydroxide using methyl red as indicator. Results are given in table -7.

TABLE - 7

Time (in hours)	Reading with blanks (in ml)	Volume of alisali used (in hi)	Corresponding amount of formic acid liberated in any	Total formic acid liberated in mg
	0.0	1.12	0,4683	13.11
16	0.0	1.20	0.5018	14.05
24	0.0	1.34	0.5603	15.69
36	0.0	1.46	0.6105	17.09
48	0.0	1.60	0.6690	18.73
60	0.0	1.70	0.7109	19.90
72	0.0	1.74	0.7276	20.37
84	0.0	1.74	0.7276	20.37

The data shows that 0.1476 moles formic acid was liberated (72 hours) per 100 gm of the polyseccharide. The amount of formic acid liberated (72 hours) corresponds to 21.69 % of anhydrohasose and pentose units present as end groups. The titre value of alkali at 48,60, and 72 hours indicated that one mole of formic acid liberated per 735.91g. 693.48g and 677.5g of the polysaccharide respectively.

vas taken out, acidified with 20 sulphuric acid (5 ml) and then 10% potassium iodide (4 ml) was added to it. The liberated iodine was titrated immediately against 10 sodium thiosulphate solution without using starch as indicator till the solution become colourless. The solution was concentrated to 10 ml to which 20 sulphuric acid (10 ml) was added and the hydrolysis was carried out for 16 hours on a water-bath. The hydrolysis was neutralised with barium carbonate, filtered and the filtrate was concentrated to a syrup under reduced pressure. The syrup was estamined by paper chromatomy graphy using different solvents the chromatograms revealed the presence of arabinose.

IX-21(b) Consumption of Metaperiodate 92

11.0

The polysaccharide (300 mg) was dissolved in water (70 ml) to which 0.25 M sodium metaperiodate (40 ml) was added and the total value of it was made upto 120 ml with water. A blank was also prepared with 0.25 M sodium metaperiodate (40 ml) diluted to 120 ml with water. The periodate ofidation was carried out at room temperature. 2.0 ml aliquots were withdrawn from the reaction mixture and blank at various intervals and 20% potassium iodide solution(2 ml) was added followed by addition of 0.5M sulpuric acid (3 ml). The liberated iodine was titrated immediately against 0.0404 M sodium thiosulphate solution using starch as indicator. The reading with the polysaccharide were substructed from the corresponding reading of control experiment to get the titre

Trians	Yolume of hour) hypo used (in ml)	corresponding amount of pariodate consumed (in mg)	Total periodate consumed (im mg)
8	1.18	5.100	306.01
16	1,32	5.705	342.32
24	1.42	6.137	360.25
36	1.50	6.483	399,00
48	1.60	6.915	434.94
60	1.68	7.261	435.68
72	1.72	7.434	446-06
84	1.72	7.436	446.06

The amount of metaperiodate consumed (72 hours) corresponds to the consumption of 0.6947 moles periodate per 100 g of polysaccharide. After 72 hours periodate cuidised solution (10 ml) was hydrolysed with 2M sulphuric acid. The hydrolysate was examined chromatographically for the presence of D-galactose, D-xylose and L-arabinose. The chromatogram showed the absence of all the three sugars.

II. 22 PARTIAL ACID HYDROLYSIS OF POLISACCHARIDE

The polysaccharide (7 g) was suspended in water (500 ml) in a three necked flask and was dissolved stirring mechanically. The hydrolysis was carried for four hours at 30° by adding 0.26 hydrochloric acid (5 ml) and the solution was stirred throughout the process. The contents, after cooling down at room temperature were poured in ethanol (2 litres) to precipitate the degraded polysaccharide. The precipitate was filtered and washed well with ethanol. The filtrate and washing were neutrilised with silver carbonate

with stirring. The precipitate was filtered, washed with water and combined solutions were concentrated under reduced pressure to a syrup.

II. 22.1 Beamingtion of the precipitate

The precipitate was hydrolysed with 2N sulphuric acid for 18 hours, over a water-bath. The hydrolysate was cooled, neutralised with barium carbonate and filtered. The filtrate and washings were concentrated and examined chromatographically over Whatman No.1 filter paper using solvents (A) and (C). The chromatograms showed three spots corresponding to R values of D-galactose. D-sylose and L-arabinose which was confirmed by co-chromatography with their authentic samples. Due to small amount of precipitate, further studies were not possible.

II.22.2 Examination of the hydrolysate

The hydrolysate was examined paper chromatographically using solvents (A),(B),(C) and (G). The chromatograms showed seven spots on spraying with aniline hydrogen phthalate and drying at 120°, indicating the presence of seven sugars.

11.22.3 Separation of Gligosaccharides

The syrup was dissolved in minimum quantity of water and applied on twenty sheets of Whatman No.3 paper as long thin band, three inches below the upper end and one inch away from the outer edges. Each paper has three guide strips, two on outer edges and one in the centre. After developing the paper on solvent (8), for sixty hours, they

were dried. The guide strips were cut from the chromatograms, sprayed with aniline hydrogen phthalate and dried at 120° with the help of the guide strips appropriate sections were cut from the unaprayed portion of the chromatograms and sugars were eluted with water. In all, seven fractions were obtained.

II.22.4 Stamination of Fraction I and identification of

12-B-xylosylaylobiose (C. F.D-xylopyranosyl(1->3)-0-F.D
xylopyranosyle(1->4)-D-xylopyranose).

This fraction was crystallised from ethanol, m.p. 223° [<] $^{21}_{D}$ = 50° (in water, C, 2.7%). Nylotriose values were 1.38 and 1.45 in solvents (F) and (B). R values in solvent (F) and (B) were found 0.73 and 0.25 respectively.

The complete acid hydrolysis with 2M sulphuric acid subsequent neutralisation with barium carbonate and examination by paper chromatography indicated the presence of mylese only, which was further confirmed by co-chromatography with an authentic sample. The molecular weight of the sugar was found to be 420 by hypoiodite method 50 which corresponds to trisaccharide of pentose units. Molecular weight calculated for $C_{15}H_{26}O_{13}$, 414.

Partial seld hydrolysis of trisaccharide with 0.5M hydrochloric seld at 100° for 30° minutes gave mylose, mylobiose and shodymendolose. Periodate emidation studies revealed that one mole of the pligosaccharide consumed 4.3 moles of metapariodate and 2.1 of formic seld liberated. I also confirmed the presence of 1-3 linkage between two

xylose units in cligosaccharide molecule.

The augar was completly hydrolysed with emals in. suggesting the presence of β - linkage. From the above observations the augar was identified to be -0- β -D-Mylo-pyranosyl-(1->3)-0- β -D-Mylopyranosyl (1->4)-D-Mylopyranose i.e. $3^2 + \beta$ -1-crylosylmylobiose. The constants of augar are given below in table -9.

TABLE - 9

Constants	Pound	Reported	References	
Mopo	223	2250	(93)	
Optical rotation	区] 21 -50°	[K] 22 -52°-	-47 (93)	
		* 10		
Rylotrione in solvents(F) &(B)	1.38 6 1.45	1.36 4	(93)	
A values in solvents(F) &(B)	0.73. 0.25	9.72, 9.20	(93)	

II.22.5 Braminetion of Fraction II and identification of Phodymenabiose

"kylobiose values were 1.97 and 1.02 in solvents(3) and (F) respectively. Recrystalised from methanol, m.p. 196° . $\boxed{\times}_{2}^{22}$. 21° (in water, C, 2.91%).

Acid hydrolysis of the sugar with 20 sulphuric acid and neutralisation of the hydrolystate with barium carbonate followed by paper chromatography in solvent (C), reveals that the presence of mylose only. The molecular weight was determined by hypoiodite mathod 20, molecular weight calculated for myloses, Castala 22.

The periodate exidation studies showed the consumption of 3.24 moles of metaperiodate with the liberation of 1.18 moles of formic acid. The augar was completely hydrolysed with emulsin, showing the presence of β -linkage. The identity was further confirmed by preparing its phenyl osazone derivative, m.p. 198-199°, $\left[4\right]_{\rm B}^{22}$ +47° (in pyridine, c.2.2%), and calculated for ${\rm C_{22}H_{28}Q_yH_4}$, N, 12.18% found 12.30% constants of sugar were compared with those reported in literature shown in table 10.

TABLE - 10

Sugar or Derivative	Constants		Reported	An Eostan Eo
Rhodymeneb Lose	(A.)2+	2920	192-93°	(94)
Rholymenabiose	kylobicse in solvent	1.97	1.97	(95)
40 B	(B) Optical rotation	[K] 23 -31°	(K) ₂₂ - 18.	(95)
	$\begin{array}{cccccccccccccccccccccccccccccccccccc$		4-> = 0.60	
3-0D-orylopyra-	MoD	196-99 [©]	194-96	(94)
nesyl-D-Xylose- phenyl esazone				
-00-	Optical rotation	[K] D +47°	[x] #7°	(94)

II.22.6 Bramination of fraction III and identification of Xylobiose

The fraction was recrystallised from agenous ethanol, map. $187-98^{\circ}$. [\times] $_{\rm D}^{20}-26.4^{\circ}$ (in water, C, 3.5%). R values were in solvents (8) and (7) 0.30 and 0.86 respectively.

Hydrolysis of the sugar with 2N sulphuric acid and neutralisation of the hydrolysate with barium carbonate

followed by paper chromatography in solvent (C), revealed the presence of mylose only which was further confirmed by co-chromatography with an authentic sample. The molecular weight of the sugar was 298, calculated for C10 H18 9, 282.

The periodate oxidation of sugar consumed 4.31 moles of metaperiodate with the liberation of 2.21 moles of formic acid indicating the $(1\rightarrow4)$ linkage between xylose units. The oligosaccharide completely hydrolysed with emalsin indicating the 7s -linkage between two units.

Thus the oligosaccharide is a disaccharide composed of Daxylone linked through -7 -glycosidic bond. The sugar was identified 4-6-7-D-mylopyranosyl-Daxylone, which was confirmed by preparing the phonyl ossione derivative, maps 205° and $[\times]_{D}^{25} = 52^{\circ}$ (in pyridine : ethanol).

The constants of sugar are given in table - 11.

Table - II

Sugar or derivative	Constants	Found	Reported	ang er		
Wylobiose			185°,187°		0.0	-
en Ĉiĝen	optical [x	Jp -26-6	区] 20 -25,	(96)
			[x] 20-32°-	→ (98,97	")
-do-	a to	D-30	→ 25.5° 0.33	•	96)
Phenyl Gaasone		205 [©]	205°	(96)
-do-	Optical [K]	25 -52	[x] -50°	(96)

II.22.7 Bramination of Fraction IV and identification of G.B.D.Galactopyranosyl(1.)41.0.B.D.Wlopyranose 52.96

The fraction was recrystalised from methanol having the optical rotation $\left[\times\right]_{D}^{39} + 1.5^{\circ}$ (in water) m.p. 190-92°. Acid hydrolysis with 20 sulphuric acid and neutralisation of the hydrolysate with barium carbonate, followed by paper chromatography, rowaled the presence of D-galactose and D-xylone. The quantitative estimation by the method of Hirst and Jones 43 showed the molar ratio 1:1 between two sugars in the oligosaccharids.

Periodate oxidation studies showed the consumption of 4.35 moles of periodate and liberated 2.1 moles of formic adid.

Mathylation of the disaccharide and followed by acid hydrolysis of the fully mathylated derivative afforded 2,3,4,6, tetra-0-mathyl D-galactone and 2,3-di-0-mathyl D-mylose in equal proportions. The oligosaccharide was completely hydrolysed with emulsin indicating the presence of β -linkage between the two units. These results proved that oligosaccharide was $4 - 0-\beta$ -D-galactopyganosyl-D-mylopyranose.

II.22.8 Reminstion of Fraction V and identification of 3-0-4-D-sylopyranomyl-learabinose.

Syrup. $\left[\times\right]_{D}^{25}$ + 172-160° (in water), R in solvent (R) 0.48, Molecular weight of the sugar was 398, calculated for $c_{10}^{H}_{18}^{O}$,282. The sugar was hydrolysed with 20 sulphuric acid and neutralized the hydrolysets with barium cambonate.

followed by paper chromatography revealed the presence of Daylose and Larabinose. The quantitative estimation by the method due to Hirst and Jones 43 showed the malar ratio between two sugars in the oligosaccharide to be 1:1.

Periodate oxidation studies showed the consumption of 3.25 moles of periodate with the liberation of 1.2 moles of formic acid.

Mathylation studies of the oligosaccharide followed by acid hydrolysis of the fully mathylated derivative of oligosaccharide afforded 2.3.4-tri-mathyl-D-wylose and 2-0-mathyl L-arebinose in equal proportions. The oligosaccharide was not hydrolysed with emulsin indicating the presence of Linkage between the two units. The results proved that oligosaccharide was 3-0-6-cD-sylopyranosyl- L-arabinose.

The constants of sugar are given in table-12.

TABLE - 12

Syrup of sugar	Pomá	Reported	Asferances
R in solvent(R)	0.49	0.49	(61)
Optical rotation	[K] 25+172-	→ [x] ²⁵ +175°-	→ (61)
		→ 180°	

11.22.9 Stamination of Fraction VI and identification of

The sugar was hydrolysed with 20 sulphuric acid, followed by neutralisation of hydrolysate with berium -

earbonate and paper chromatography, revealed the presence of D-galactose units only. The molecular weight was found to be 347 calculated for $c_{12} c_{22} c_{11}$, 342 by hypotodite method, which corresponded to a disaccharide of homose units.

Periodate exidation studies showed the consumption of 4.22 moles of matapariodate with the liberation of 2.18 moles of formic acid.

Mathylation study of the oligosaccharide followed by acid hydrolysis of fully methylated eligosaccharide afforded 2,3,4,6-tetra-G-methyla-D-galactose and 2,3,4-tri-G-methyla-D-galactose and 2,3,4-tri-G-methyla-D-galactose which was transformed by bromine oridation in alkaline solution afforded formaldehyde 64. It was concluded that the biose linkage was 1->4 type. Oligosaccharide was not hydrolysed by hydrolysis with emulsis, it follows that the linkage was <--type.

The above observations identified the oligosaccharide to be 4-0- \times -D-galactopyranosyl-D-galactope.

XI. 22.10 Stamination of Fraction VII and identification of Learnbinose.

M.P. and m.m.p., 156°, $[<]_{D}^{29} + 104°$ (in water, C.1.26%) Lit. m.p. 150°, $[<]_{D}^{29} + 101°$.

The sugar (.2 gm) gave phenyl osazone on heating with phenyl hydrazine hydrocheride (0.4 gm), crystalline sodium acetate (0.6 gm) and water (6 ml) on a boiling water-bath for 30 minutes. The solution was cooled and the precipitated phenyl osazone was filtered and recrystallised from agencys ethanol, map, and mamp, with an authentic sample 1632164 75

May Bloom Care

- 1. Duthie, J.F.; 'Flora of the Upper Gangetic Plain',
 Vol. 1 P. 154, (1960), Copyright by the Government
 of Radia.
- Chopga, RyNy, IsChopga, IsCy, and Nayar, Syleys
 Chossary of Indian Medicinal plants', page No. 262, (1956)
- Sanderman, W., Distarichs, H.H. and Gottwald, A. 1
 HolzRoh-4, Herb-staff, 16, 197 -204 (1958).
- 4. Chakrabarty, P.K.; Indian J. Med. Research, 23.
- 5. Franch, R.B. and Abbott, O.D., Florida Agr. Mapt.
 Sta. Tachn. Bull. , 444 , 21, (1948).
- 6. Back, K. M. Lam, S. Y., Han, D. J., Rim, J.J., You gu Nommanjip, Chunchon Nonghaa Tambak, 2, 21-4 (1969).
- 7. Yenko, F.M.; Baens, L., West, Augustus, P. and Curran. H.A.; Phillippine J., Sci., 47, 343-48 (1932).
- 8. Simmeini, 8., Boll. Stam. Spar. Ind. pell Hat. conciantio, 16 , 173-82 , { 1938 }.
- 9. Tandon, P.; Arya, H.C.; Stperientia, 36 (8), 958-9, (8ngg.) (1980)
- 10. Woo, Wun Sicks Shin, Nuk Hyung Kang, Sam Siks Racamb.

 Adv. Nat. Prod. Res. Proc. Int. Symp., (Pub. 1980)

 33-40 (Eng.) (1979).
- 11. Rosa, J.S. and Ichan, A., Anais Assoc. quim. Brasit.,
 10 , 236-53 (1951).
- 12. Antonaccio, L.D. ; New, Quim. Ind. (Riode Jameiro).
 26 , 126 (1957).
- 13. Harbhajan Singh, Seshadri, T.R. and Subramanian, G.B.V.

- CHER. Sci. 34. (11), 344-45 (1961).
- 14. Rajadurai and Theresa, M.Y., Leather Sci. 10 (5)
- 15. Rec, V.S., Sudraj Suddy, X.K., Sastry, K. . and Nayudammaa Y.; Leather Sci., 15 (7), 189-93 (1968).
- 16. Manard, E.L.; Mueller, J.M., Thomas, A.F.; Ehatnagar, S.S. and Dastoor, N.J.; Helv. Chem. Acta, 46, 1801-11 (1963).
- 17. Matthias, P. Maslinger, M., Mairel, M., Monatch. Chems 100 (5) 1608-12 (1968).
- 18. Techescha, R., Wilhelm, H., Fehlhaber, H. W.;
 Tetrahadron Lett., 26, 2609-12 (1972).
- 19. Srivastava, S.K. Srivastava, S.D., Phytochem., 18 (10), 1758-9 (1979). (Sng.).
- 20. Tomoda, M., Askura, H., Hida, A.; Sayakugaku Zasshi, 23 (2), 45-48, (1969).
- 21. Gkamura, Nobuyuki; Nobara, Toshihiro; Yogi, Akira; Nishioka, Etsuo; Chem. Pharm. Bull 29 (3), 676-83 (1981), (2ng.).
- 22. Manda, P.C., Dutta, B.K., Jodha, M.R., Scie, Cult., 36 (5), 286-88 (1970).
- 23. Bisko, J. And Zellner, J.; Monatach, 64 , 12-16, (1934).
- 24. Noo, Non, Sicks Kang, Sam Sik, Shim, Sang Myuck, Manger, Hildebert, Chari, Vedantha Mohan Seligmann, Otto, Georgeter, Guenther, Soul Tuchakkyo Saongyak Yonguso Opyakjip, 18, 17-19, (1979) (Reg.).
- 25. Moo, Non, Siefer Tang, Sam Sik, Shim, Sang Hyucke Manger, Hildsberk, Cheri, V.Moham; Saligmann, Octor

- Chermeter, Guenther, Phytochemistry (1979).18 (2). 3535 (Eng.).
- 26. Ikram, My Ogihara, Yy Yamasaki, K.; J. Nat. Prod. 44 (1), 91-3 (1981) (Eng.).
- 27. Teran, E.N., Farmetsiya, 4, No.11/12, 20-23 (1941).
- 28. Tang, Tang-Han and Chao, Yuan-Haiang; J. Chinese Chem. Sec. 4 , 278-86 (1936).
- 29. Kawaguti, and Kim, K.W., J. Pharm. Soc., <u>60</u>, 595-6, Abstract 236-36 (1940), (in sing.).
- 30. Majumdar, B.; Sarkar, S.N and Dutta, P.C.; J. Ind. Chem. Sec. 33 , 351-52 (1956).
- 31. Akhmedov., VA . and Khalmakov., Kh.Kh.; Formatsiya,
 16 (3), 34-35,(1967).
- 32. Chaghtoi, M.I. D., Mokhar, Izshad, Fasselot, Tahira, F Pak. J. Sci., 30 , (1-6), 136-44, (1978).
- 33. Admedov, U.A. and Malmatov, Ma.Kh; Polz, Dikoma**rtuschie** Rast. Uzb. Eth. Tspol *sedi *Fan*. Uzb. S.SB. 154-8.(1968).
- 34. Alchmedov, V.A. and Khalmatov. Kh.; Rast Resur; 5 (4), 579-81 (1969).
- 35. Mehta, T.N., Rao, C.V.N. and Laumikantam, V., India Soap. J. 19 , 44-45 (1953).
- 36. Blouch, A.K., Hijjatullah, S.; Sci., Bea.; g, (1-2),1-6, (1969).
- 37. Shibate, S. Negi, Y., Tanaka, O., Doi, O. Phyto chemiskry.
 2 .(3) 677 (1970).
- 38. Mous, O. Oghara, Y. Yamaski, K.J. Chem. Ras. (5), 4 . 144: 144-8 (1978).
- 39. Shanmugavelu, K.G. Rangaswami, G.; J. Sci., Cult., 35 (10), 581-82 (1969).

- 40. Ahmad, Moghis, V., Hasain, S.K., Ansari, A.A.,

 Osman, S.M., J. Oil. Technol. Assoc. India 11 (3),

 70-2, (1979).(Sng.).
- 41. Tschesche, Mudolf, Shah, Arif H.; Sckhardt.; Phytochemistry, <u>18</u> (4), 702-4, (1979), (Mg.).
- 42. Airan, J.W., Rajopdhya, S.B., J. Indian Chem. Soc., Ind. and News Rd., 12 , 152-54 (1949).
- 43. Hirst, E.L. and Jones, J.K. N.; J. Chem. Soc., 1659, (1949).
- 44. Parik, V.M., Ingle, T.R. and Bhide, S.V., J. Ind. Chem. Soc., 35, 125 (1958).
- 45. Purdie, T. and Ervine, J.C., J. Chem. Soc., 83 ,1021(1903).
- 46. Cill, R.E., Hirst, E.L. and Jones, J.K.N., J. Chem. Soc., 1025 (1946).
- 47. Hampton, H.A., Haworth, W.N., and Hirst, E.L., J. Cham.
- 48. Haworth, W.W. Raistrick, M., and Stacey, M., Biochem., J., 29, 2668 (1935).
- 49. (a) Oldham, Mary A., and Honeyman, J., J. Chem. Soc., 98 6 (1946).
 - (b) Hirst, E. L. and Jones, J.K.N. J. Chem. Soc., 1221 (1947)
- 50. Higst, E.L. Hough, L. and Jones, J.K.W., J. Chem. Soc., 928 (1949).
- 51. Cerezo, A.S., J. Org. Chem., 30 , 924 (1965).
- 52. Montgomery R., Smith, P. and Srivastava, H.C.; J. Am. Chem. Soc., 29, 698 (1957).
- 53. Mikes, 0.; 'Leboratory Hand-book of Chromatographic Methods', Ist Ed. (Eng.).; Van Hostrand, P.71 (1966).

- 54. Rizvi, S.A.I.; D.Phil. Thesis, University of Allahabad, India (1968).
- 55. Andrews, P., Hough, L. and Jones, J.K.N., J. Am.
 C hem. Soc. 74 , 4029 (1952).
- 56. Hamilton, J.K., Parlow, E.V. and Thomson, N_1S_{11} J. Am. Cham. Soc.; S2 , 451, (1960).
- 57. Aspinall, G.O., Rashbrook, R.B. and Keesler, G., J. Cham. Soc., 215 (1958).
- 58. Maier, H.; Acta Chem. Scand., 14 , 749 (1960).
- 59. Andrew, P. Hough, L. and Jones, J.K.M., J. Chem. Soc., 2744 (1952).
- 60. Rirst, E.L. and Jones, J.K.N., Dicuss, Faraday, Soc., 7 . 268 (1949).
- 61. McFerren, E.F. Kathleen Brand and Batkowski, M.R., Anal. Cham., 23 , 1146 (1951).
- 62. (a) Tewari, S.N.; J. Anal. Chem., 176, 604 (1960).
 (b) Wilson, C.M.; Anal. Chem., 31, 1199 (1959).
- 63. (a) Marier, J.R., Sonlet, M.; J Dairy Sci., 42, 1390(1959)
 (b) Dubois, M., Cilles, K.A., Hamilton, J.K., Rebers,

P.A., Smith, F., Anal. Chem., 28 , 350, (1956).

- 64. Schryvar, S.B. Proc. Roy. Soc. (London) B, 82,226 (1910).
- 65. whistler, R.L., and Comrad, M.E., J. Amer. Chem. Soc., 76, 1673 (1954).
- 66. (a) Lederer, S. and Lederer, M.; " Chromatography", Elsvier's P. 166, (1995).
 - (b) Mikes, O., "Laboratory Hand-book of Chromategraphic Mathods, Ist Ed. (Sng.). P. 88 (1966).

- 67. Trevelyan, 2-2-s Proctor, D.P. and Harrison, J.S.s. Hature, 166, 444, (1930).
- 68. Belcher, R., Fildes, J.E. and Nutten, A.J.; Analyt. Chem. Acta, <u>13</u>, 16, (1955).
- 69. Belcher, R. and Godbert, A.L., 'Semi-micro Quantitative Organic Analysis', 2nd Ed., P. 164, (1954).
- 70. Barker, S.A., Foster, A.M., Siddigui, I.R. and Stacey, M.: Talanta, 1 , 216 (1938).
- 71. Partridge, 5., Biochem. J., 42 , 238 (1948).
- 72. Master, L., 'Methods in carbohydrate chemistry'; Blitor
 Royl, L. Whistler, Academic Press, Inc., Vol. II, P. 117,
 (1963).
- 73. Hisaki, A. and Smith, F.; Agr. Food. Chem., 10 ,104,(1962).
- 74. Mukherjees and Srivastava, H.C. J. Am. Chem. Soc., 77 , 422, (1955).
- 75. Stephen, A.M., J. Chem. Soc., 4487, (1956).
- 76. Pastuska, G., J. Anal. Chem., 179, 427 (1961).
- 77. Smith, F. and Montgomery, R., The Chemistry of plant Game and Macilages', American Chemical Society Monograph Series, Reinhold Publishing Corporation, New York, P. 134 (1959).
- 78. Souveng, H.O., Kieseling, H., Lindberg, B. and Mckay, J.E., Acta Chem. Scand., 16, 615, (1962).
- 79. 'Chromatographic Analysis' Comeral Discussion, Faraday Soc., 7, (1949).
- 80. Garegg, P.J. and Lindberg, B., Acta. Chem. scand.,
 14 , 871, (1960).
- 81. Percival, E.G.V. and Willow, I.C., J. Chem. Scci., 1608 (1949).

- 82. Robertson, G.J., Speedie, T.H., J. Chem.Soc., 824, (1934).
- 83. Chanda, S.K., Hirst, R.L., Jones, J.K.M., Pegcival, E.G.V., J. Cham. Soc., 1289 (1950).
- 84. Hampton, H.A., Haworth, W.H. and Hirst, E.L., J. Chem., Soc. 1739 (1929).
- 85. Ethrenthal, S., Rafique, M.C., and Smith, F., J.Chem. Soc., <u>74</u>, 1341 (1952).
- 86. Hirst, E.L., Percival, E.G.V. and mylam, C.B.; J.Cham. Soc., 189, (1954).
- 87. White, E.V. and Rao, P.S., J. Am Chem. Soc., 75, 2617(1953).
- 88. Brown, F., Halsall, T.G., Hirst, E.L., and Jones, J.K.M.,
- 89. Invine, J.C. McNicoll, D., Ibid., 97, 1449, (1916).
- 90. Cifonelli, J.A. and Smith, F.; Anal, Chem., 26, 1132.
 (1954); Ebid., 77, 1984 (1935).
- 91. Cyong, Jyong-chyul; Ham abusa, Kiyomichicoviant, Mad.

 Res. cent. Kitasato Inst, Tokyo, Japan, 108,

 Phytochemistry, 19 (12), 2747-8 (1980). (Smg.)
- 92. Hough, L. and Powell, P.B.; J. Chem. Soc., 16 (1960).
- 93. Whistler, Releg Ta, C.C.; J. Amer. Chem. Soc. 74.
- 94. Curtis, S.J.C., Jones, J.K.M.; Cand J. Chem., 39 ,1305, (1960).
- 95. Haward B.H.; Blochemical J., <u>67</u>. 643 (1957).
- 96. Srivastava, H.C. and Smith, F. J. Am. Cham. Soc. 72. 982, (1957)

97. Whistler, R.L., Bachrach, J. and Tu, Chem-chuan; J. Am. Chem. Soc., 74, 3059 (1952).

98. Whistler, R.L., Tu, C.C., J. Am. Chem. Soc., 72, 1389, (1951).

CHAPTER - III

A WATER SOLUBLE NEUTRAL POLISACCHARIDE

FROM THE UNRIPE PRUTES OF

MISA SAPISATUM LINN.

III.1. The present Chapter describes the isolation and structural elucidation of a water soluble neutral polysaccharide from the unripe fruits of <u>Mass sepientum Linn</u>e belongs to the family Musaccas¹.

The plant Muda sapientum Linn. is commonly known as Kela (Samana). A tropical fruit, having its origin in the Malayan penunsula, the 'Samana' an African name, is one of the cheapest and most liked mutritive fruit. This is one of the most popular and widely grown plant of the tropics and subgropics, produced in enormous quantities for expert. The plant is considered symbolic of prosperity. There are numerous varieties of Samana differing in size, shape, colour, flavour of fruit and suitability for handing and expert.

In India, all Banana called Plantain² but the word is usually used for the large fruited, starchy or cooking variety important for human food in many countries especially tropical Africa, these belongs to the species Phase paradisiacs while most eating varieties belongs to species <u>Phase sapientum</u>.

in length with a thickness of 1 to 1.5 inches (2.5 to 3.5 cms). Some variaties have a thick skin while others a thin skin. The skin may be yellow or red. The fruit has a whitish sweet pulp with agreeable flavour. The plant is indigenous in Bihar and the Eastern Himalayas upto 4000 ft. cultivated throughout India.

and vitamins with a high calorific value. Demulcent and astringent properties are attributed to them. Popularly used in distellic treatment of sprue, chronic dysentries and diarrhoses. Ripe fruit is Peels of green bananes are useful as dermatol therapentic agent against e.g. ecsema, skin cruetion, chaps or burn and as skin creem against wrinkle 106. Unripe fruit is astringent and ripe fruit is anticorbutic used as a mild demulcent, astringent, dist in cases of dysentery 1.

diarrhoga and dysentery and promutes the healing of intestinal lesions in ulcerative colitis. Ripe fruit is also useful in diabetes, uramia, nephritis, gout, hypertension and cardiac diseases. The stalk of the fruited plant is given to pigs in Shina for kidnery worms. The ash of the root or of the whole plant is anthelmintie.

The work done in the past years on this genus was surveyed and the details of it are given in the tabular forms

Gunu		Spacios	Constituents	Parts	Reference
1.	Bonona		Determination of Apeorbic acid (Vitamin C)		(1936) ³
2-	Sanana	****	Riboflavin content (Vitamin B ₂)		(1941)4
3.	Banana	dia	Vitamin A, C and Vitamin B-Complex		(1946) ⁵
4.	Banana	499	xanthophylls, xryto- xanthin, lycopene, neolycopene, neo-B- carotene-U, B- carotene, neo-B- carotene-B, K- carotene (carotenoid pigments).		(1949)
5.	Banana		Composition of Ascor- bic acid content		(1950)7
6.	Sanana (Ta bra	•	Separation & charact- erization of fructosy sucrose		(1963) ⁶
7.	Amona (Talua		Partial hydrolysis of fructosyl sucross with \$P_D_fructofuranoside yielded fructose, glucose & a reducing disaccharide (on further hydrolysis yielded glucose & sucrose) Enzymetic hydrolysis yielded sonosecharide reside sonosecharide reside		
8.	Banana		Biochemistry, Physiolo & Nutritive Value of Banana-Analysis of	97	(1968)10

COUD	Spacios	Constituents	Parks B	Kerence
		emino acid, carbohydgate minegal, tannin, organic acid		
		Proteins & fats, vitamins		
		(escept for 8 ₂) starch content(20-32%).		
9. Bana	路根 🖜	cycloeucalenol, cycloarte- nol & 24-mathylene cyclo- artanol, esters of palmatic acid, stigmasterol, camp esterol, & B - sitosterol	Rhizome, stalk and leaves	(1969) ¹¹
10. Bana	na •	Rutin, quepoetinglyco-	•	(1970)22
		ehin, ferulic acid, chlo-		
		rogenie acid, p-coumaryl quinic acid, cinnamic acid		
		and derivatives, dopa, novadrenalime, dopamine, lewecanthogynidin, 5- hydroxy trytophan, L-try-tophan, 5-hydroxy tryto-mine & trytomine.		
11. Bana	ana om	Physiochemical nature of Banana pseudostem starch (Amylase content)	600 >	(19 70)¹¹
12. Sand (Ta	ima Lwan)	Determination of carbo- hydrate(Galactose.gala-	Skin and akin inte	
		ctouronic acid, glucose, arabinose, fructose and Thamnose in polysaccha-	riors and	
		ride),starch1.85%, soluble starch & destrin		
		%.glucose 4.69% and gructose 1.16%		

Cenus	8	enten	Const. Struents	Parts	Refugates
13. Ba			Pelagonidin, Cyanidin, Peonidin, delphinidin, Pefunidin, malvidin,	ägadis	(1954) ¹⁵
14. Ba	mana	***************************************	(Anthocyanins) Malanins	Buds and flowers	(1967) ¹⁴
15. B	nan4	****	Glutamic acid	Skin of Danana	(1959) ¹⁷
16. B	anana	€D-	Clucose, fructose, sugrose from the alco- hlie extract of Banana	Green, rij and perio rp of fr	
17. B			Dopamine	Pruits	(1964)19
10. a		gin	Sucrose, glucose and fructose	Edible part of fruits	(1967) ²⁰
19. B	anana	400	Constitution of Samana starch(on hydrolysis affords 97% glucose	Unripo Erwika	(1940) ²¹
20. 8	anana.	400	Extraction, purification and amplace content of green banana content of green banana contain 25%	Green Banana	(1971) ²²
21. B	anana		S-hydroxy tryptamine, phonyl Butazone.	Unr ipe banana	(1964)23
and the same	anana Solumbi	en All	Chemical & Biochemical characterisation of	Ripe & green	(1970) ²⁶
b	anana)		Danana. It is a source of starch.Protein, minerals(K.Mg.Ca.Fe.Mn, Ma) tannin.pectins, disaccharide.alkaloids, & fiber content.	peel	

Gert u.S	Species	Constituents		(oranga)
23. Banena		Long chain free fatty aldehyde	Lipid	(1979) ²⁵
14. Banana	200b	Maltose, glucose, suc- rose, fructose & 3 or 4 other sugars.	Green & ripe pulp	(1955) ²⁶
5. Janana		Citric acid, L-Malic acid, (Organic acids)	Crean & ripe Sanana	(1954) ²⁷
6. Banana	48h	Dopa, Dopamine, sero- tonin, Indole-3- acetic acid & normpl- nephrine	Peel	(1963) ²⁸
7. Banana (2-Varie- ties of	₩	Vitamin B	Ripe	(2943) ²⁹
Havana) 18. Banana	400	Vitamin A.Band C	Ripe	(1937)30
39. Banana	4699	Lipid, 18 fatty acids C-C chain between 6- 22, unsaturated fatty acid of 16-18C	Ripe peel & pulp	(1969)31
10. Danana	4008	Alkanoic acid, C nos (C6-C22) 4-unsatura- ted acid, palmatic acid	perleary	
31. Benana		Noradrenaline, sero- tenin, 3-(3,4,-dihydro- xy phenyl) amine (Phenantolamine)	Pulp and	22
32. Benana		An entacid, demalcent,	, Pulp	(1965) ³⁴
33. Masa	Sapion- tun	Antibectorial substances(Antible= tie)	Leaves	(1949) ³⁸

C enti	19	Species	Constituents 1	(a.g.)	Reference
34.	Musa	Sapien-	Capito (3-3-01- A-sterol) & several derivator ACdi Brdi-H.and B-Cap		(1955) ³⁶
	Мали	Sapien-	"Ho n-alkanes from C ₁₉ "30, several substituted nuphthales, phenanth- erenes & related agomatics. (Aromatic hydrocarbon).	las	(1966) ³⁹
36.	Masa	Sapion-	Hypoglycenie substan- ces	Flowers	(1965) ³⁸
37 •	Mass	Sapien-	Triterpene katone (31-noggyelolaudamone)	Pool	(1970)39
38.	Maa	Sapien- tum		Paal	(1970) ⁴⁰
39.	lèssa	Accual-	A new Laugoanthocyani-	Soad	(1962)41
40.	M103	Accumi-	Proenthocyanidia glycosida	Locks	(1971)42
41.	Masa	nata Ca ven - dishii	Analysis of oligo- saccharide by the examination of paper chromatography of 80% aq. StoH Strack.		(1964) ⁴³
42.	Masa	Caven- dishii	fructose, maltose, mylose, Raff inose,	and stalk	(1965) ⁴⁴
43.	, same	Caven- dishii dearf	Mamose, mannose Malie, citrie, phos- phorie acids	balb	(1963) ⁴⁵

7

(2 varioties of China)

Sapati I		Spectos	Constituents	Parts !	No September 2018
)4 ₀		Paradio-	Vitamin A & B		(1927)46
5.	(Porto	iaca prico plantai:	n)		
5.	Miss	Paradis-	Vitamin B(B,)		(1930)67
		taca	or G		
16.	Maga	Paradia-	D-glucitol	Leaves	(1966) 48
		isca	(Sorbitol)		
7.	Maga	Paradis-	Detraction of	Seeds	(1929)49
		laca	pactina & mueilage contant.		
48.	Masa		Catacholamine &	Various	(1968) ⁵⁰
			serot in in (Mono-	organs & tissus	
49.	Maga	(Payllium)	Pharmeognosy	•	(1934) ⁵¹

from different plant products as have already been described in literature, but no neutral polysaccharide has been mentioned on the unripe fruits of Musa sepientum Linna uptill now.

Therefore an attempt has been made for isolation and structural elucidation of the polysaccharide from the unripe fruit of this important plant Masa sapiantum.

III.2 STRUCTURAL BLUCTDATION OF NEUTRAL NATER SOLIBLE POLYSACCHARDS PROM THE UNKIDS PRUITS OF MEA SAPIRITIES

III.2.1 RESULE AND DECESION

A new water soluble polysaecharide has been isoluted

from the defatted unripe fruits of M.saplentum, by extracting with 1% acetic acid and precipitating with excess of ethanol. The polysaccharide was repeatedly purified till the ash content reduced in minimum. The homogeneity of the polysaccharide checked by :

- (1) Fractional precipitation,
- (ii) 20ne electrophoresis,
- (111) Acetylation and deactylation.

separated into three fractions by fractional precipitation with different volume of ethanol. All the three samples were analysed quantitatively by the method of Hirst and Jones 54. The results were essentially identical to the original polysaccharide indicating the polysaccharide to be homogenous.

The portion of the polysaccharide was separated by zone - electrophoresis method in borate buffer (pH 9.3).

After completion of the experiment, a plot of the absorbance against segment numbers showed only a single sharp peak indicating the polysaccharide to be homogeneous.

The homogeneous polysaccharde was acetylated with acetic anhydride and sodium acetate. The acetylated product showed optical rotation $\left[\times \right]_{D}^{25} + 29.5^{\circ}$ (in chloroform,C, 0.85%). On descetylation, it gave a polysaccharide having the same optical activity as the original one. Thus it confirmed the homogeneity of the polysaccharide.

IXI.2.2 The polysaccharide was slowly soluble in water,

[<] $_{\rm D}^{25}$ 473.6° (in water, C,0.8%), ash content 0.75%. The polysaccharide was found to be free of nitrogen, sulphur, and halogens. The methodyl, uronide and acetyl percentage were found to be negligible.

with 28 sulphuric acid followed by paper chromatographic analysis of the hydrolysate revealed the presence of three sugars, D-galactose, D-mannose and D-mylose. The identity of the sugars was confirmed by their specific optical rotations, preparation of their crystalline derivatives and co-chromatography with authentic samples.

The quantitative estimation of the mono-saccharide components by periodate oxidation taking Ribose as a reference sugar showed that mannose, galactose and xylose are present in the molar ratio 4:1:1 in the polysaccharide.

O.058 sulphuric acid and subsequent paper chromatographic analysis of the hydrolysate taking out at various intervals, revealed that galactose was liberated first followed by the liberation of mylose and mannose respectively. This shows that mannose units are linked together forming the backbone (main chain) of the polysaccharide and galactose and most of the mylose units are linked as terminal groups. The easy liberation of galactose units indicate that most probably they are linked to the main chain at peripheri

III.4 The polysaccharide was methylated first by Haworth's

method using dimethyl sulphate and alkali⁵⁵ followed by Purdie's method⁵⁶ giving a methylated polysaccharide having optical activity [4]_B²⁵+40° (in chloroform,C,1.5%) =0CH₃.

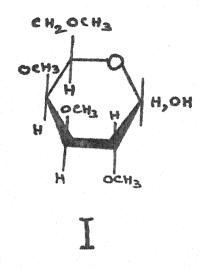
0. 44.06%. The complete hydrolysis of the methylated polysaccharide and paper chromatographic analysis of the hydrolysate in solvent (A), revealed the presence of five methylated sugars. The methylated sugars were separated on a preparative scale by chromatography on Whatman No.3 filter paper. The following methylated sugars were identified.

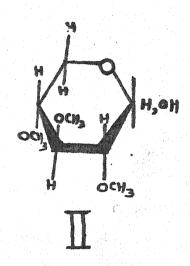
- (1) 2,3,4,6-tetra-G-methyl-D-galactose;
- (2) 2,3,4 -tri-0-methyl-Decyloges
- (3) 2-0-mathyl-1-xylosar
- (4) 2,3-di-Gampthyl-D-Mannose;
- (5) 2,3,6-tgi-G-mathyl-D-mannose.

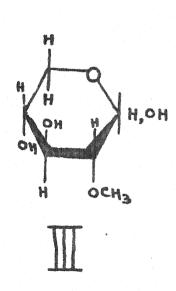
Methylated sugar (1), had R_{IMG} in solvent(A), 0.89, mop. 71-73° [<] $^{25}_{D}$ +123° (in water,C, 0.5%). On treatment with ethanolic aniline gave 2,3,4,6-tetra-0-mathyl-N-phenyl-D-galactosylamine, mop. 188-89°, [<] $^{25}_{D}$ -80° (in acetome, C,1.1%). Therefore, the identify of the methylated sugar 1, is established as 2,3,4,6-tetra-0-mathyl-D-galactose.

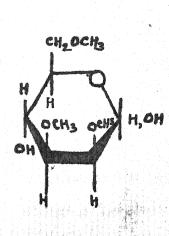
Mathylated sugar (2), was obtained as a syrup could not be recrystallised, $R_{\rm TMS}$ in solvent (A), 0.95, $\left[\left\langle \right\rangle \right]_{\rm D}^{18}$ + 19.2° (in water, C, 0.38). On treatment with ethanolic aniline it gave, 2,3,4-tri-0-mathyl-D-xylopyranosyl anilide, m.p. 95-96°, $\left[\left\langle \right\rangle \right]_{\rm D}^{22}$ - 60° (in ethanol, C, 2.5%). The sugar in this fraction was thus identified as 2,3,4-tri-0-mathyl-D-xylose.

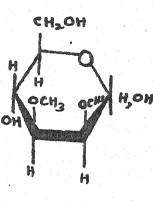
Mathylated Sugar (3), had Rpms value in solvent(A),











V

0.39, $\left[\times \right]_{D}^{25}$ =25° (in water,C, 2.3%), m.p. 132-33°. It formed 2.0-methyl-D-mylose anilide, m.p. 122-24° $\left[\times \right]_{D}^{25}$ +210° (in ethylacetate,C,0.9%). Its discetate, 2.0-methyl, 3.4-discetate had m.p., 76-77°, $\left[\times \right]_{D}^{25}$ = 39° (in chloroform,C, 3.0%). Thus the above observations confirmed that the methylated sugars, 1 is 2-0-methyl-D-mylose.

Mothylated sugar (4), was also obtained as a syrup, R_{TMG} in solvent (A) 0.154, $\left[\times \right]_{D}^{26} = 16.6^{\circ} (\text{C, 1.8 \% in})$ water). It formed 1,4,6, p-nitrobenzoate with p-nitrobenzoyl chloride, m.p. 190-92° $\left[\times \right]_{D}^{26} + 63^{\circ}$ (in chloroform C,1.5%) which shows that the methylated sugar (4) is 2,3-di-0-methyl-D-Nannosa.

Mathylated sugar (5), R_{TMS} in solvent(A), 0.82, [] 25_12.5° (in water, C, 1.6 %) formed 1.4-bis-p-nitrobensoate, m.p. 186-87° [] 36 +32° (in chloroform, C, 0.5 %). On exidation with bromine water, it gave a lactone, which on treatment with phenyl-hydrazine f-ormed 2,3,6-tri-G-methyl-D-Mannonic acid phenyl hydrazide, m.p. 128-30°. This indicates that the methylated sugar (5), is 2,3,6-tri-G-mothyl-D-Mannose.

The quantitative estimation of methylated sugars by the method of Hirst and $Jones^{57}$ showed that sugars 1,2,3,4,5 were present in the molecular ratio, 2:1:1:2:6.

The studies indicate that galactose units in the polysaccharide occupy terminal positions as nonreducing end groups from which 2,3,4,6-tetra-O-methyl-D-galactose (1), arises on hydrolysis of the methylated polysaccharide. A

large portion of (5), 2,3,6-tri-0-mathyl-D-mannose(6 moles) indicates that the backbone of the polysaccharide consists of mannose units linked through 1-4 linkages. Detection of 2,3,-di-0-mathyl-D-mannose (2 moles) shows that two mannose units in the main chain per repeating unit of the polysaccharide are linked at position 6 in addition to -1 and -4 - positions. Isolation of 2-0-mathyl-D-mylose(Imole) made an idea that one mole of mylose unit per repeating unit of the polysaccharide is linked at position 1,3,4- Presence of 2,3,4-tri-0-mathyl-D-mylose (1 mole) shows that one mylose unit in the polysaccharide occupy terminal position through 1-3 linkage.

Determination of terminal groups by periodate emidation and subsequent titration of liberated formic acid, corresponds to 0.782 moles of formic acid per 100 g of the polysaccharide, is supposed to consist of 12 sugar moleties of which 2 units of galactoss and one unit of mylose form terminal groups. Considering such a repeating unit, the terminal groups were found 24.91% as determined by periodate emidation studies which is identical to that revealed by methylation studies (25.03%).

Ilias The partial acid hydrolysis of the polysaccharide followed by paper chromatographic separation on preparative scale afforded five (5) oligosaccharide. The following oligosaccharide were detected t

- 1. Mannotriose, Q-P-D-mannopyranosyl(1->4)-Q-P-D-mannopyranosyl (1->4)-D-mannopyranose.
- 2. Spinslibiose. 6-0- (-D-galackopyranosyl-b-

Fig - 1

512-2

- 3. 6²- < -galactosyl mannobiose, 0, < -D-galactopyranosyl (1→6) -0-B -D-mannopyranosyl-(1→4)D-Mannopyranose.
- 4. Mannobiose, 4. Q.B. D. Mannopyranosyl. D. Mannopyranose.
- 5. Rhodymanabiose, (0- β -D-xylopyranosyl(1->3)-0. β -D-xylopyranose).

oligosaccharida (1), m.p. 168-69°, [] 18.8°(in water, C, 1.8%) was chromatographically pure in solvent (C), (G) and (F). It was shown to be monehydrate of trisaccharide on the basis of its equivalent weight, 264.8. Acid hydrolysis of the oligosaccharide yielded only mannose. Fartial acid hydrolysis yielded mannose and mannoblose which were identified by co-chromatography with the authentic samples. The identity was also confirmed by the periodate oxidation data which showed the liberation of 2.10 moles of formic acid with the consumption of 5.3 moles of meta periodate per moles of sugar. Hence the oligosaccharide was identified to be =0-B -D-manno pyranosyl-(1-4)-0-B -D-mann

oligosaccharide (2), was isolated in exystalline form having the physical constants identiful with those reported for 6-0- \langle win-galactopyranosyl-D-mannopyranose. It reduced Fahling solution and Tollen's reagent having map. 200-02°, $[\langle \rangle]_{D}^{32} + 120.5^{\circ}$ (in water, C.C.45 %) and was found to be a single entity by paper chromatography in three different solvent systems (A), (B) and (C). The paper

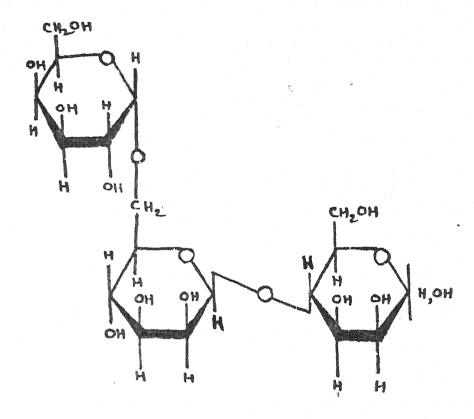
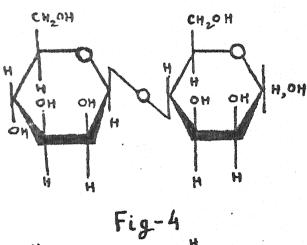


Fig-3



chromatographic analysis of the completely hydrolysed sugar revealed the presence of galactose and mannose. The quantitative estimation by the method of Mirst and Jones 56 showed the molar ratio 1 : 1 between the two sugars in the oligosaccharide. The equivalent weight, 174.2, showed the to be a disaccharide. The periodate oxidation studies afforded the liberation of 3.2 moles of formic acid and consumption of 5.24 moles of periodate per mole of disaccharide. The liberation of 3.2 moles of formic acid from the disaccharide indicates that there is 1-6 linkage between galactose and mannose units. As the disaccharide could not be hydrolysed with emulsin, it is inferred that calactose and mannose have <-linkage between them. On the basis of above evidences, the oligosaccharide was identified to be epimalibiose, 6-0-<-Degalactopyranosyl-Dmannopyranose and identity was further confirmed by cochromatography with an authentic sample. (Fig. 2).

Oligosaccharide (3), was crystallised from ethanol, m.p. $226-27^{\circ}$, $[<]_{D}^{32}+98.5^{\circ}$ (in water,C, 0.5%). It was shown to be a single entity by paper chromatography in solvent (0), (c) and (3). (Fage 94). It reduced Fehling solution and Tollen's reagent. The complete acid hydrolysis of sugar and subsequent paper chromatographic examination revealed the presence of galactose and mannose. The quantitative estimation by the method of Hirst and Jones 54 showed that galactose and mannose are present in the oligosaccharide in the ratio 1:2. The equivalent weight, 262.8, showed it to be a trisaccharide. The periodate exidation studies showed the liberation of 3.18 moles of

periodate. Partial acid hydrolysis followed by paper chromatographic examination showed the presence of manno-biose and epimelibiose besides galectose and mannose. Their identity were confirmed by co-chromatography with authentic samples. The oligosecharide was, thus identified as O-K - D-galactopyranosyl (1->6)-O-73-D-mannopyranosyl-(1->4)-D-mannopyranosyl-(1->4)-D-mannopyranose. (Fig. 3).

Oligosaccharide (5), a caystalline sugar, n.p. 191° , $[\times]_{p}^{22} - 21^{\circ}$ (in water, C, 2.92 %), was found to chromategraphically pure in the solvents(F) and (a). The sugar on acid hydrolysis yielded only xylose while the solecular weight of the sugar 266 corresponded to a pentose disaccharide. Enzymic hydrolysis with emulsin showed the

presence of \$\begin{align*} = \text{linkage between two mylose units. The periodate omidation showed the consumption of 3.26 moles of of metaperiodate with the liberation of 1.18 moles of formic acid per mole of the sugar. The identity was confirmed by co-chromatography with an authentic sample. The oligosaccharide is, therefore identified to be \$\mathbb{C}_7^2 = \mathbb{D}_{\text{mylopyranose}}\$. (Rhodymana-biose). (Fig. 5).

III.7 On the basis of the results obtained so far particularly from the methylation studies, graded and partial acid hydrolysis, the following valuable informations could be derived.

- (i) The main chain of the polysaccharide consists of B - (1→4) linked mannose units.
- (11) One xylose unit per repeating unit of the polysecharide is also linked in the main chain through $\beta = (1 \rightarrow 4)$ linkage.
- (111) Galactose units are linked in side chain to the main chain through $\kappa = (1-6)$ linkages.
 - (iv) One mylose unit is present in the side chain through β -linkage, position, 1,3, and 4.
 - (v) $B = (1 \rightarrow 3)$ linkage between xylose and xylose units is present in the side chain only.

Taking all the experimental evidences into consideration together with the structures of different oligo-secharide, the following most probable structure has been assigned to the polysaccharide from the fruits of Miss.

$$- \left[4 - \frac{1}{16} - \frac$$

Gal@ = Galactopyranosa

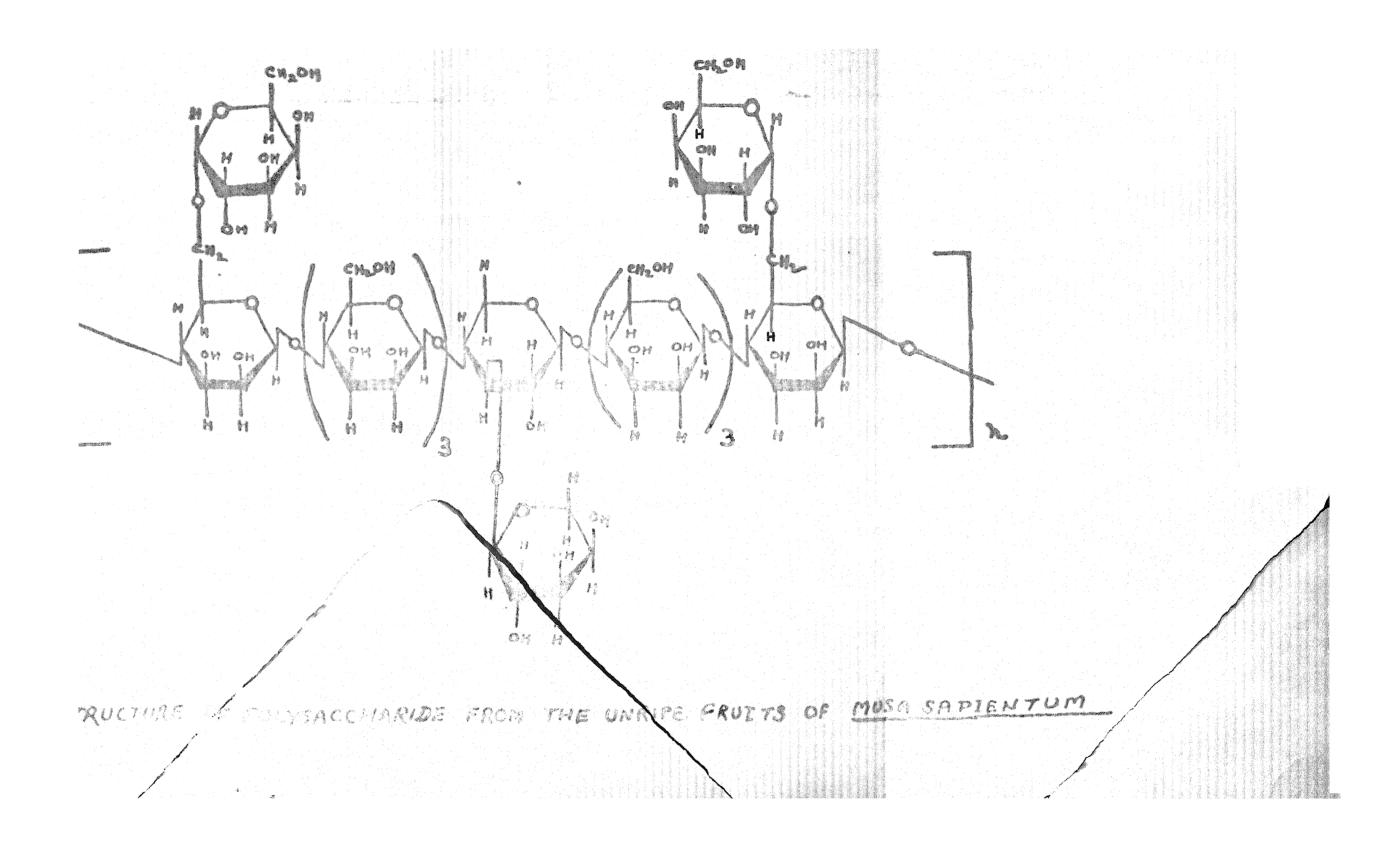
MylP m Mylopyranose

ManP = Mannopyranose.

and pentose monosacch; les per repeating unit, which fully explains the formation of oligosaccharides as obtained by partial acid hydrolysis and agrees well with the analytical data of the polysaccharide. The dotted line and doubly arrowed lines show the probable mole of fission of linkages during the partial acid hydrolysis. The arrowed dotted lines indicate secondary hydrolysis.

The polysaccharide such as described above should consume 14 moles of metaperiodate with the liberation of 3 moles of formic acid per repeating unit of 12 sugar units. The actual consumption of periodate and liberation of formic acid have been determined to be 14.15 moles and 2.98 respectively per repeating units of polysaccharide which are in close agreement to the calculated values.

Possibility of the similar structure cannot be completely ruled out but they are less probable because the formation of oligosaccharide as obtained in the present case might not be possible.



III.8 EXPERIMENTAL

Departmental techniques were same as described on (Page 34). Paper chromatography was performed at room temperature by descenting technique on Whatman No.1 filter paper unless stated otherwise using following solvent systems:

(A)	n-Butanol-ethanol-water	(5:1:4)59
(3)	n-Butanol-acetic acid-water	(4:1:5)59
(c)	n-Sutanol-isopropanol-water	(11:1:5)60
(D)	Benzene-ethanol-water	(169:47:15) ⁶¹
(2)	Butanone - Water	(10:1)62
(P)	sthylacetate - pyridine-water	(10:4:3)63
(G)	Sthyl acetate-pyridine-water	(2:1:2)64
(H)	n-Butanol-gthanol-water	(40:11:19)65
(I)	n-Butanol-pyridine-water	(6:4:3)66

The spots were located by spraying the chromatogram with aniline hydrogen phthalate⁶⁷ and heating it at 120° for 10-15 minutes. Spectrometric determination wars carried out by a modification of phenol-sulphuric acid method⁶⁸. Klett-Summerson photoelectric colorimeter was used for measuring the absorbance.

III.9 BOLATION OF THE POLESACCHARDS

The dried and grushed unripe fruits (3 Kgs) were extracted successfully with petroleum ether (60-80°) and ethanol. The polysaccharide extracted from the extracted unripe fruits of Musa sapientum by the repeating the process as given on page 35. A colourless fibrous precipitate of the crude polysaccharide was obtained. It was filtered, washed

with absolute ethanol and dried in vacuum at room temperature (49 g, ash content 3.15%).

TIL 10 PURIFICATION OF THE POLYBACCHARIDE

The dried crude polysaccharide was dissolved in distilled water (2 litres) containing 1% acetic acid with constant stirring. The solution was filtered and was added very slowly to ethanol (8 litres) with constant stirring and kept over-night. The precipitated polysaccharide was filtered and the above process was repeated four times, to get a white fibrous mucilage, (35.8 g. ash 0.75%).

INT. 11 HOMOGENERAY OF THE POLESACCHARIDE

The homogeneity of the polysaccharide was chacked by the following methods.

III.11 (a) Fractional Precipitation

The pure mucilage (Sg) was fractionally precipitated into two fractions (Fraction I and Fraction II). Both the fractions alongwith the original polysaccharide were hydrolysed and quantitatively estimated by the usual method as described on page 44. The ratio of mannose, galactose and kyloge in both the fractions was found almost the same(4:1:1) indicating the purified polysaccharide was to be homogeneous.

III.11 (b) Zone - electropheresis

Polysaccharide (300 mp) was taken for Zone-electrophoresis and similar procedure was adopted as described on Page 38.

The corrected absendance reading (Table-1) are as follows, so obtained wars platted against the distance from

the anode, that is segment number which showed only one sharp peak indicating the polysacharide to be homogeneous.

TABLE - 1

iumber number	eluka	Blank Klett reading	Corrected Klett reading	Ab gogbands
1	 26	28	1.0	0.003
	27	25	2.0	0.004
3	26	24	2.0	0.004
4	28	25	3.0	0.006
2 3 4 5	27	25	2.0	0.004
6	25	23	2.0	0.004
7	25	21	4.0	0.008
6	25	22	3.0	0.005
9	24	22	2.0	0.004
10	24	23	1.0	0.001
11	25	22	3.0	0.006
12	30	25	5.0	0.010
13	28	25	3.0	0.006
14	35	33	2.0	0.006
15	37	21	16.0	0.032
16	50	21	29.0	0.058
17	38	21	17.0	0.036
10	23	26	3.0	0.006
19	25	27	2.0	0.004
20	25	24	1.0	0.002
21	25	21	4.0	0-008
22	25	21	4.0	0.008
23	24	22	2.0	0.004
24	24	23	3.0	0.006
25	28	25	3.0	0.006
26	28	22	6.0	0.012
27	27	25	2.0	0.004
	23	21	2.0	0.004
28		21	4.0	0.008
29 30	25 25	22	3.0	0.006

Absorbance was measured on 5 ml portion of coloured solution.

Absorbance = 2 x Klett reading .

III.11 (c) Acetylation and Deacetylation

The pure mucilage (2g) was mixed thoroughly with anhydrous sodium scatate (30 g) and the mixture was suspended in acotic anhydride (30 ml) and further process was repeated as on page 37. The acetylated polysaccharide (1.5g) was obtained having the optical rotation $\begin{bmatrix} \times \end{bmatrix}_D^{25}$ +29.5 (in chloroform, C,0.85%).

The dried acetylated polysaccharide (1.0 g) was dissolved in acetone (30 ml) and 50% potassium hydroxide solution (30 ml) was added to it. The deacetylation was carried in the usual manner 69 , as given on page 38 . The deacetylated polysaccharide (0.5g) having $\left[\times\right]_{D}^{25}$ + 73.5° (in water, C,0.78 %) with close agreement to original one indicating the homogeneity of the polysaccharide.

ITI. 12 ASH CUNTENT

The dried polysaccharide (0.4g) was ignited in a silica erucible which previously heated to a constant weight.

After ignition, the crucible cooled in a desicator and weighed. From the weight of residue (0.0014 g), the ash content was calculated 0.75%.

III. 13 PHYSICAL AND CHEMICAL EXAMINATION

It was a fibrous white powder, very light in weight, slowly soluble in water, $\left[\kappa\right]_{\Sigma}^{25}$, 73.6° (in water, C.G.85%). For the purpose of optical rotation, the solution was filtered through a sintered glass funcel to get a clear solution and the amount of polysaccharide in the solution was determined colorimetrically. The polysaccharide was found to be free

from nitrogen, sulphur and halogens. On treatment with Fehling's solution, it formed an insoluble copper complex but did not reduce it.

TIT. 14 EXAMINATION OF PRICE SUGARS

The polysaccharide was examined for free sugars by applying three spots of its solution in a water on a strip of Whatman No.1 filter paper (15 x45cms) and developed in solvent (A) as described on page 40. The three spray reagents naphtharesorcinol and trichloroacetic scid and aniline hydrogen phthalate and silver nitrate in acetome followed by ethanolic sodium hydroxide on three different strips of above paper showed no spot, hence it showed that the polysaccharide was free of any free sugar.

III. 15 METHOKEL GROUPS DETERADIATION

The percentage of methoxyl groups was determined by the method of Belcher, Fildes and Nutten 72 and was found to be negligible.

III. 16 ACETYL GROUPS DETERMINATION

The method by Belcher and Godbert 73 was followed for the determination of acetyl group percentage with and without mucilage which was found in significant (0.96%).

III. 17 URGNIDS CONTENTS DETERMENATION

The uronide contents were found to be negligible by the semi-micro method of Baker, Foster, Siddiqui and Stacey 74.

III. 18 HEDROLES IS OF POLES ACCHARDS AND DETERMINATION

OF MONOSACCHARIDE

The purified murilage (1.5 g) was dissolved in 2%

sulphurie acid (100 mL) and was hydrolysed on a water-bath for about 24 hours. The hydrolysed polysaccharide was neutra-lised with barium carbonata, filtered and concentrated under reduced pressure. The hydrolysed was examined paper chromato-graphically for monosaccharides.

TII.18 (a) Paper Chromatography

of Whatman No.1 filter paper. The papers were developed separately in solvents (A) and (B) by descending unidimensional technique. The chromatograms were six-dried and sprayed with aniline hydrogen pathalate. On heating them is an oven at 120° each chromatogram showed three spots. The R_g and R_G values of the three spots corresponded to Demannose, Degalactose and Demylose as given in the table ~2.

TABLE - 2

Sugar	Sol	vent (A)	30176	et (B)
ident if ied	R G Lound	RG 75 Given 75	R _g found	Rg given 59
D-Manage	0.12	0.11	0.20	0-31
D-Calactose	0.06	0.07	0.17	0.16
D-Xylose	0.14	0.15	0.27	0.28

G = 2,3,4,6-tetra-0-mathyl-D-glucose.

The identity of the three sugars was further confirmed by co-chromatography with authentic sample of the sugars in the same solvent systems.

III.18 (b) Column Chromatography

A portion of hydrolysate was dissolved in a small

amount of aquous methynol(1:1) and absorbed over a column of cellulose (2: 35 cms). The column was left over-might and the separation was effected with solvent (A). Fractions amounting to 10 ml were collected and checked by paper chromatography with authentic samples of D-mannose, D-galactose and D-mylose in solvent (B). The fractions 1-12 containing same sugar were combined together and concentrated to give D-mannose. It was recrystallised from equous methanol, [x] $\frac{25}{D}$ + 12.9° (in water, C, 1.7 of par 100 ml of solution). The following two derivatives were prepared:

(1) D-Mannose Phenyl hydragone

Found

Givan⁷⁶(Lit.). 199-200°

(11) D-Mannose panaglycosylamino benzoic acie

The derivative was prepared according to the recent method of ${\rm Ellis}^{77}$.

Pound

Given (Lit.)77

map. 179-181°

The fraction 16-25 were mixed and concentrated to give 1-galactoss. It was recrystallised from equeous methanol, $[4J_{\rm B}^{25}+77^{\circ}]$ (in water,C. 0.5g per 100 ml of solution). The

following derivatives were prepared :

(1) D-Galactose Phenyl Hydrazing

Found

Given (Lit.)78

m.p. 154 -155

154 -155

(11) N-p-nitrophanyl-D-Galactsylamine

In a micro test-tube, galactose (45 mg), p-nitroeniline (45 mg), one drop of glacial scalic ecid and four drops of methanol-water(8:1 v/v) were taken. The misture was boiled for 8 minutes and kept over-night in a refrigerator. The crystalline product was filtered, washed with cold sthanol, ether and dried in vacuum. It melted at 217-18° after recrystallisation from methanol. Lit 79 m.p. 219°.

The fraction 30-38 containing same sugar were combined together and concentrated to give D-sylone. It was recrystallised from aquous methanol, $\left[\times \right]_D^{30} + 18.5^{\circ}$ in water, c, 1.15 %), m.p. 144-45°. The following derivative was prepared.

(i) Desylose phenyl caszone derivative

The ogazone of the sugar was prepared as given on page 43 m.p. 160-161° resembling to an authentic sample.

III.18 (c) Thin-layer chromatouraphy

The plates were prepared from slurry of silica Gal G in O.1M solution of boric acid and the spots of hydrolysate alongwith benseneracetic acid : methanol (1:1:3) and air dried. These plates were sprayed with aniline hydrogen phthalate reagent on heating them at 120 in an oven three spots corresponding to D-galactose, D-mylose and D-mannose were observed.

III.19 DUANTITATIVE ESTIMATION OF MONOSACCHARIDE

The polyeaccharide (200 mg) was hydrolysed with 2M sulphuric acid (35 ml) for 24 hours on a boiling water-bath and neutralised with barium carbonate. Albose (20 mg) was added to it. The hydrolysate was applied on whatman No.1 filter paper alongwith the guide strip. After developing in solvent (c), the strips corresponding to the sugars wave cut

with the help of guide spots and eluated. The eluate was omidised with periodate and the quantity of the monosaccharide estimated as described on page.

CASES ...

							(into
Sugar	volum	n of all	talio	Corres	pending	account.	
		da (m)			ar (in		de la constante
	A		G	Δ.			district
		A 60	2.76				ordenine sec.
D-Calactose	3.36	4.22	4070	0.97	1.21	0.00	
D=Nannoso	13,60	16.98	11116	3.92	4.89	3.21	
D=39 1,050	3.18	4.03	0-62	0.98	1.20	0.79	
D-Albosa	1.96	2.42	1.60	0.59	0.73	0,49	

* Strangth of sodium hydroxide = N/124.8.

Assuming complete recovery of D-Ribose the above results indicate that in the polysaccharide, D-mannose, D-galactose and D-Hylose in the molar ratio of 4:1:1.

III.20 GRADED HEDROLYSIS OF THE POLISACCHARIDE

The polysaccharide (150 mg) was dissolved in 0.05-N-sulphuric acid (30 ml). The hydrolysis carried out over a boiling water-bath. The hydrolysite, taken out at various intervals, were estamined chromatographically, without removal of sulphuric acid using solvent (8) for the purpose of igrigation of the paper. Results are given in the table -4.

TABLE - 4

Timo	Suguar identified	Ho.of other
(in minutas)	Galactose (Faint)	
15	Galactose exylose (Faint) Galactose + Xylose	
45	Galactose + Wlose	Two spoks Three spoks
₩	Galactose+Wylose + Mannose(Very faint)	
	Galactose + Xylose + Humose (Faint)	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
		· · · · · · · · · · · · · · · · · · ·

Time (in minutes)	Sugar Mont 16 ted)			`	No.of spoks	ol.hag	
180	Galactose minnose	*	Mylose	•			COLO.	
240	Galectose mannose	•	Xy lose	•		Three	spoks	

Degalactors was found to liberate first followed by the liberation of Degalactors and Demannoss. The easy release of Degalactors leads to the conclusion that galactors is present as terminal group, and not in the main chain of the polysaccharide. The release of Degalactors before the release of Demannose shows that most of mylose units are present as terminal group and mannose units are present in the main chain and formed the backbone of the polysaccharide.

III.21 MEDITLATION OF THE POLISACCHARIDE

The polysaccharide (10g) was dissolved in minimum quantity of water and was methylated first by the method due to Parilde. Ingle and shide 55 followed by Purdie's mathod and usual describes on page 47.

The partly mathylated product was brownish mass(7.8g), - 0CH_3 , 36.4%, $[\propto]_D^{25}$ +55.6° (in chloroform,C.1.5 per 100 ml of solution). The partly mathylated polysaccharide was further methylated by Purdie's method as given on page 48. The fully methylated polysaccharide was obtained as a deep brownish coloured product (6.8 g) found-OCH₃, 44.06 $[\propto]_D^{25}$ +40° (in chloroform,C,1.5 g per 100 ml of solution).

DENVIRATION OF MARKETS SUGAR

The hydrolysis of methylated polysaccharide was

earried by slight modification of method due to Houveng etcal 62 . The methylated polyseccharide (100 mg) was dissolved in 85% formic acid (20 ml) and rest of the process was carried out as described on page 49.

After separation on whatman No.1 filter paper in solvent (A), the chloroform chromatogram of syrup showed five spots after spraying with antiline hydrogen phthalate and drying at 120°. The R_{IMG} value of each methylated sugar was calculated in solvent (A) and was compared with that, given in literature as shown in the following table -5.

TABLE - 5.

Methylated sugars identified	R _{TMS} Sound	vent (A) R _{TMG} 58, 83 given
2,3,4,6-Tetra-Compthyl-Dogalactose	0.90	0.88
2.3.4.tri-O-mathyl-Dacylose	0.98	0.94
2m Omitiest by Landonsky Luses	0.39	0.38
2,3 -di-O-mathyl-D-mannose	0.45	0.44
2, 3, 6-tri-Comethyl-Commence	0.82	0.81

III. 23 QUANTITATIVE ESTIMATION OF METRICLATED SUGARS

III.23.1 The methylated polysaccharids (300 mg) was hydrolysad as given above. To the hydrolysate glucose (60 mg) was added and then neutralized with barium carbonate.

The chromatogram wase developed by descending method using solvent (D) as described on page 94.

machod as given on so 50 the desults detained are given

Table 6

. 6800 · ·	ection 6 3 y	luma (lod (ir	of C.IN	hypo		onding a	
					A		69-
λo	2,3,4,6-tetra- 0-methyl-B- galactose	0.60	1.28	0.94	0,872	1.395	1.024
D.	2,3,4,trimOm methylmDm xylose	0,50	0.80	0.60	0,435	0.696	0.522
C.	2-6-methyl-De xylose	0.60	0.96	0.70	0.438	0.700	0.511
D.	2.3.dim O-meth- yl-D-mannose	0.92	1.46	1.10	0.874	1.387	1.045
Ŝ.	2,3,6-tri-0- mathyl-D- mannose	2.56	4.12	3,00	2.611	4.202	3,006
P.	@uccee	0.58	0.94	0.70	0,522	0.846	0,633

The above results corresponded to an average malazer to between A, B, C, D and E as 2:1:1:2:6. The mathylated sugars were calculated as the mathyl ethers of anhydrohomose and pentose units, i.e. $C_6H_{12}O_5$ and $C_8H_{16}O_5$ for monomand trim 0-methylates and $C_{10}H_{20}O_5$. $C_9H_{18}O_5$ and $C_8H_{16}O_5$ for monomand totra—, trim and dim-0-methyl sugars respectively. An average recovery of the methylates polysaccharide was found to be 98.88% assuming 100% recovery of glucose.

III.23.2 CHARACTERES ATTON OF METHYLATED SUGARS

The methylated polysaccharide was hydrolysed according to the method of Garege and Lindberg as described on page 52. The mixture of different methylated sugars was resolved into five fractions on Whatman No.3 filter paper using solvent(D) Strips containing different individual methylated sugars were eluted with water. The elumina were concentrated sugars separately under reduced pressure and marked as fractions I.

II, III, IV and V.

III.23.3 Fraction I

solid, R_{TMS} in solvent (A), 0.89 , found GM_{B} S1.3% calculated for tetramethyl herose, GCH_3 , 52.54 %, $\left[\times \right]_D^{25} + 123^6$ (in water,C,0.5%), Lit. 85 for 2,3,4,6-tetra-G-mathyl-D-galactose $\left[\times \right]_D^{16} + 142^6 \rightarrow +117^6$ (equil.) (in water,C,1.1%), map. $70-72^6$. It gave red colour with aniline hydrogen phthalate. Its treatment with alcoholic aniline gave 2,3,4,6-tetra-G-mathyl-N-phenyl-D-galactosylamine, n.p. $188-89^6$.

TIL. 23.4 Praction II

Syrup, it could not be recrystallised. The $R_{\rm TMS}$ in solvent (A) 0.95, optical rotation of sugar was found to be $[\ll]_{\rm D}^{18}$ + 19.2 (in water, C, 0.38 %), Lie 4 is $[\ll]_{\rm D}^{15}$ + 203°, ONe found 54.98 %, calculated for $C_8^{\rm H}_{16}^{\rm O}_5$ is 55.35%)

The anilide of the sugar was prepared as given on page 53 The malting point of the anilide was found to be 95-96°, $[<]_{B}^{22}$ 80° (in ethanol.C.1.3%) Lit. 86, m.p. 120° [$<]_{D}^{\circ}$ 84 \rightarrow > 47° (in ethanol) and Lit. 87 m.p. 91°. The mathoxyl value of the derived anilide was found to be 33.6% ($C_{14}H_{21}^{\circ}$) N requires, 0% 34.3%).

The sugar in this fraction was thus identified as 2,3,4-tri-0-methyl-D-cylose.

III.23.5 Fraction III

Solid, R_{2100} in solvent (A), 0.35, ON_{2} , 18.92% calculated for some mathyl pentose, C_{1}^{2} , ON_{2} , ON_{2} , 18.90 %, m.p. 132-133°, C_{1}^{2} C_{2}^{2} C_{3}^{2} C_{4}^{2} C_{4}^{2} C_{5}^{2} C_{5}^{2}

[$\[\]_D = 23 \rightarrow + 35^\circ \]$ in water) Lit. mop. 132-33, [$\[\]_D = 24 \rightarrow +36^\circ \]$ in water). It formed 2-0-methyl-D-mylome smilide on treatment with ethanolic aniline, mop. 123-34°, [$\[\]_D = 10^\circ \]$ (in ethyl acetates C, 0.9 %).

On acetyletion as usual method it formed a crystalline compound 2-0-methyl-D-xylose; 3,4-di-acelate, m.p. 76-77°, $\begin{bmatrix} < \end{bmatrix}_D^{25} = 39^\circ \text{ (in chloroform, C, 3.0%) Lit.}^{99} \text{ m.p. 78-79}^\circ \begin{bmatrix} < \end{bmatrix}_D^{90}$ (in chloroform). Thus the above confirmed that the methylated sugar III, is 2-0-methyl-D-xylose.

III. 23.6 Fraction IV

Syrup, R_{TMG} in solvent (A), 0.54, $[<]_D^{26}$ -16.6° (in water, C, 1.8%), found ONs, 29.37% calculated for dimethyls CMs, 29.8%, $[<]_D^{-}$ 16.0° (water) in it. 90.

The sugar (100 mg) was dissolved in pyridime. It was finally washed with water and dissolved in chloroform. The insoluble portion was filtered out and the solvent from the filtrate was evaporated in a vacuum desicator. The crude product was recrystallised from ether, m.p. 190-192, [K]D + 63° (in chloroform, C. 1.5%). Lit 81, for 1.4,6-p-nitrombensoate of 2.3-di-0-methyl-D-mannose, m.p. 194° and [K]D 165° (in chloroform).

III. 23.7 Fraction V

Syrup, R_{TMG} in solvent (A), 0.82, found CMs, 41.3% calculated for tri-mathyl hexases CMs 41.9% $\left[< \right]_D^{25} = 12.5^{\circ}$ (in water, C, 1.6 g per 100 al of solution). Lik 92 for 2,3,6-tri-G-mannose, $\left[< \right]_D^{25} = 10^{\circ}$ in water.

The syrup (100 mg) was dissolved in dry priding (6 ml)

and treated with p-nitrobenzoyl chloride (35.0 mg) for 45 minutes at 60-70° and left over-night at room temperature. A saturated solution of sodium bicarbonate was added dropwise until no effervescene occured. After adding water (15 ml), the product was extracted with chloroform. The extract was dried over sodium sulphate, excess of solvent was taken off in vacuum and erystallised from petroldum ether, m.p. 186-870, $\left[< \right]_{D}^{26} + 32^{\circ}$ (in chloroform, c, 0.5%). Lik 93,94 gor 1,4-bis-pnitrobensoate of 2,3,6-pri-C-methyl-D-mannose, m.p. 187-880 and [<] p+ 33.00. The syrup (100 mg) was oxidized with bromine water and the product crystallised from aceton-petroloum other, map. 80-81° Lit 61 for 2,3,6-tri-0-mathy2-7- (+) mannolactone, m.p. 82-83°, the lactone (75 mg) was boiled under reflux in alcohol with phenyl hydrasine (45 mg). IR Was then refluxed with little amount of animal chargoal in ethenol and filtered. On cooling, a czystalline product was obtained which was recrystallised from sthanol, map. 128-30 $\left[\propto \right]_{0}^{25} = 20.5$ (in water, C, 0.8%). Lik⁹⁵, for 2,3,6-tri-0. methyl-D-mannonic acid phenyl hydrazide, m.p. 131° [4] = 20° (in water).

III.24 PERICUATE CARPATION OF THE POLESACCHARIDE III.24(a) Liberation of Formic Acid & Estimation of and groups

The polysaccharide (300 mg) was dissolved in water (50 mg) and in the solution, potassium chloride (0.5 g) and 0.25M sodium matapariodate (60 mg) were added. The volume was made upto 140 mg with water. In a blank experiment potassium chloride (0.5 g) and 0.25M sodium matapariodate (60 mg) were

diluted to 140 ml with water. The unidation was carried out in a dark at room temperature as described on page 55. The aliquote of 5 ml were taken and were titrated for liberated formic acid against N/124.8 godium hydroxide solution using methylated as indicator. Hasults are given in table - 7.

TABLE . 7

Time (in hours)	Volume of alkali used in (ml.)	Corresponding amount of formic acid (in ag)	Total formic acid (in mg)
8	1.32	0.506	14,168
16	1.40	0.536	15.03
24	1.54	0.590	16.52
36	1.72	0.659	18-46
48	1.88	0.720	20.178
60	2.00	0.766	21.46
72	2.04	0.782	21.896
80	2.08	0.782	21.694

The data shows that 0.782 mole of formic acid was
liberated (72 hours) par 100 g of the polysaccharide. The
amount of formic acid liberated (72 hours) corresponds to
24.9% of anhydrohomose and pentose units present as end
groups. The titre value of alkali at 48, 60 hours indicated
that one mole of formic acid was liberated per 683.9% and
643.05 g of the polysaccharide respectively.

III.24 (b) Consumption of Sodium Astapariodate 97

The polymercharide (300 mg) was dissolved in water (70 ml) to which 0.25M) sedium notaperiodate (40 ml) was added and the total volume was made upto 120 ml with water. A blank was also prepared with 0.25M sodium metaperiodate

(40 ml) diluted to 120 ml with water. The pariodate ontidation was carried out at room temperature as described on
page 57. The liberated indine from 2 ml aliquots of mixture
and blank were titrated against 0.0404N sodium thiosulphate
solution at various intervals using starch a-s indicator.
The reading with the polysaccharide were substructed from
the corresponding reading of controlled experiment to get
the titre values. The results are given in table - 8.

Table - 8

Time (in hours	Volume of Hypo used (in ml)	Periodate consu- med (in mg)	Total periodate consumption(in mg)
0	1.00	4,322	259.33
26	1.12	4.841	290.45
24	1.32	5.705	342.32
36	1.42	6.137	368, 25
48	1.60	6.915	414.94
60	1.72	7.434	446.06
72	1.84	7.953	447.18
84	1.06	8,039	482.36
96	1.86	8,039	482.36

The amount of periodate consumed (84 hours) corresponds to the consumption of 0.7513 moles of periodate per 100 g of the polysaccharide. After 86 hours periodate oxidised solution (10 ml) was hydrolysed with 2M sulphuric acid (Page 58). The hydrolysate examined by paper chromatographically for the presence of D-galactose, D-sylose and D-mannose bytthe chromatogram did not indicate the presence of any of the three sugars.

III. 28 PARTIAL ACID HIDRONISTS OF POINSACCHARIDE

the polysaccharide (8 g) was suspended to water

(500 ml) in a three necked flask and stirred mechanically and the same procedure was adopted as described on page 58.

III.25.1 Stamination of the Precipitate

The precipitate was hydrolysed and identified similarly as described on page 59. The chromatograms, showed three spots corresponding to R_g value of D-galactose, D-xylose and D-mannose which were confirmed by co-chromatography with their authentic samples.

111.25.2 Begainstion of the Hydrolysate

Paper chromatographic analysis of the hydrolysate over thatman No.1 filter paper using solvents (A) and (B) and aniline hydrogen phthalate as a spraying reagent produced seven spots thereby indicating the presence of seven sugars.

III.25.3 Separation of Glicosaccharide

The syrup was dissolved in minimum quantity of water.

It was separated by paper chromatography as described on page 59.

The sugars were crystallised from ethanol and five fractions of oligosaccharide and two fractions of monosaccharides were obtained.

Mannotgiose

 $R_{\rm Num~l}$ 0.07 and $R_{\rm GLM}$, 0.37 in solvent (C) and (G) respectively, $R_{\rm GLM}$, 0.21 in solvent (F). The sugar was crystallised from ethanol, m.p. 168-69° [<] $_{\rm D}$ = 18.8 (in water, C.1.5%). It reduced Fehling's solution and Tollen's reagent.

The complete acid hydrolysis with 20 sulphuric acid,

nation by paper chromatography with an authentic sample only one monosaccharide, D-mannose was obtained. The equivalent weight of the sugar was found to be 264.8 by hypoiodite method⁵⁷. Partial acid hydrolysis of sugar with 0.5% hydrochloric acid at 100° for 10 minutes resulted in formation of mannose and mannobiose which were identified by co-chromatography with their authentic samples.

periodate exidation of the sugar revealed that 2.10 moles of formic acid were liberated and 5.3 moles of periodate were consumed per mole of sugar. The sugar was completely hydrolysed with emulsin suggesting that mannose units were linked through 72 mglycosidic linkages.

on the basis of above results the sugar was identified to be mannotriose i.e. β -mannose pyranosyl- (1->4)- β -D-mannopyranose, which was further confirmed by its physical constants of it as shown in table -9.

TABLE . 9

Constants	2000	asported	References
	168 and 69	133- 139° and 214-15° (anhydrous)	(83, 98, 99)
Optical rotation	[4] 30 -18-8	[4] -15°-) - 26°	(100, 101)
R _{Glu} in	0.37	0.33	(103
and Solvent	(F) 0.21	0.22	(63, 64)

TIL-25.5 Stamination of Fraction II and Identification of Point Diose

 R_{Man} G.15, 0.26, 0.35 in solvent (A), (B) and (C) respectively. The sugar was recrystallised from ethanol, m.p. $200-02^{\circ}$, $\left[\times \right]_{D}^{32} + 120.5^{\circ}$ (in water, C, 0.49 per 100 ml of solution).

and neutralisation of the hydrolysate with barium carbonate followed by paper chromatography with solvent (c), revealed the presence of galactose and mannose in the oligosacchuride which was further confirmed by co-chromatography with an authentic sample.

The quantitative estimation by the method of Mirst and Jones 54 showed the molar ratio to be 1 : 1 between the two sugars in the oligosageharide. The equivalent weight as determined by hypotedite method 37 was found to be 174.2.

The periodate amidation studies corresponded to the consumption of 5.24 males of metapariodate and liberation of 3.2 males of formic acid per mole of the oligosaccharide. Thus there is 1->6 linkage between galactose and mannose units.

As the oligosaccharide could not be hydrolysed with emulsin it shows that galactose and mannose have < -linkage between them.

Que the basis of above observations the sugar was identified as epimeliblose, 6.0 < D-galactopyranosyl-D-mannopyranose. Its identity was further confirmed by preparing its osasone, map. 172° and co-chromatography with an authentity was property with an authentity was property with an authentity of the sugar were compared sample. The chase compared sample the chase of the sugar were compared with those resources in interacture as shown in the table.

ugas os	Constant	Found		Regerences	
erivativa Spimel Deiose	they o	200-02°	201 - 02° & 202 - 03°	(103,104)	
Epimalibiose	optical rotation	[4] 32+120-5°	[K] +130-9°	(104,105)	
	200000	(in water)	[4] +120-1		
			→124.6°(in		
			water)		
Epimel ib io sa	a _{cilu} in	0.60	0.59	(64)	
	Solvent(6)		175 - 76	(165)	
Quason@	m.P.	1720	7/9 - 10		

III.25.6 Bramingtion of Fraction III and Identification of 62 - 4 - Calactosri manchiose

R_{Mam} O.07 and O.16 in solvent (C) and (B) respectively. The sugar was recrystallised from 90% ethanol. The paper chromatography revealed only one spot, R_{Clu} in solvent (c) 0-33..m.p. 226-27° and [x] 32, 98.5° (in vater, C.0.5g per 100 ml of solution). It reduced Fehling's solution and Tollen's reagont.

The complete acid hydrolysis with 26 sulphuric acid, neutralised with barium carbonate and chromatographic examination showed the presence of galactose and magnose. The quantitative estimation by the method of Hirst and Jones 4 showed that galactose and mannose constituents the oligosaccharide in the molar ratio of 1:2. The equivalent weight was found to be 262.8 by hypolodite method ..

the particular artestan andles chartes an sole

of the oligosaccharide consumed 6.30 moles of metaperiodate and liberated 3.18 moles of formic acid. Partial acid hydrolysis rewealed the presence of mannoblose, epimeliblose besides galactose and mannose.

from the above observations, the sugar was identified to be < "Galactopyranosyl =(1->6)= β "D-mannopyranosyl=(1->4)="

TABLE __ 11

Constants	Pougad	Reported	References		
	226 - 27	228 -29	(91, 103)		
Be De			/ 64 102 3		
Optical rotation	[K] 32 + 98.5°	[K] 28 + 93-3°	(91, 103)		
		> +98,8			
R _{Gl.u} in	0.33	0,32	(66)		

Nannobiosa

in solvents(A) and (B) and (C) were found to be 0.28, 0.47 and 0.33 respectively. The sugar was recrystallised from methanol, m.p. 203° , $[<]_D^{30}$, $[<]_D^{30}$, in water, C, 1.3 g per 100 ml of solution).

Acid hydrolysis with 2M sulphuric acid, followed by neutralisation with barium carbonate and subsequent estantnation by paper chromatography showed the presence of mannose units only. The equivalent weight was detarmined by hypotodiae method 57 and was found to be 174.8.

The periodate exidation studies showed the consumption of 4.22 moles of periodate with the liberation of 2.14 makes of formic acid per mole of the sugar. The sugar was completely hydrolysed with emulsin showing the presence of β -linkage between the mannose units which was also confirmed by the negative optical rotation of the sugar.

of D-mannose units linked through B-glycosidic linkage. The sugar was identified to be mannoblose i.e. 4-0-B-ii-inannopyranose which was also confirmed by preparing osasone derivative, m.p. 205° and co-chromatography with an authentic sample. The constants of sugar are given in table - 12.

TABLE - 12

Sigar of	Constants	Poud	Reported	References
Derivative Mannobiose	i Bajo p	2038	202-204	(63,65. 83,103)
Mannob 1988	Optical [c	30 D -11-4 ⁶	[K] 70 ->-9°	(63, 83, 101, 103)
Mannob 1088	R _{Glu} in	0.52	0.52	(63, 64)
	solvent(F) and (G)	0.66	0.65	
Mann ob iosazone	Мере	205°	203-06°	(63)

III.25.8 Reminstion of Fraction V and Identification of Mhodymenabioss

Sylubiose values were 1.98 and 1.04 in solvent
(8) and (F) respectively, recrystallised from mechanol, m.p.

191°, [4] 22 21° (in water.C. 2.92 g per 100 ml of solution).

and neutralisation with barium carbonate, followed by paper chromatographic analysis in solvent (C), reveals the presence of xylose only. The molecular weight was determined by hypotodite method 57, 296, molecular weight calculated for xylobiose, $C_{10}H_{13}O_{9}$, 282.

The periodate exidation studies showed the consumption of 3.24 moles of metaperiodate with the liberation of 1.18 moles of formic acid. The sugar was completely hydrolysed with emals in showing the presence of P whinkage. Its identity was further confirmed by preparing its phenyl-osazone derivative, m.p. $197 - 98^{\circ}$, $[\times]_{D}^{22} \cdot 48^{\circ}$ (in pyridine, C, 2.0%, calculated for $C_{22}H_{28}Q_{7}H_{4}$, N 12.18, found 12.30%.

Constants of sugar were compared with those reported in literature as shown in table = 13.

TABLE - 13

SOUR SECOND THE SECOND			
contants	Pound	Reported	References
A P •	1919	192 - 93°	(53)
Reylobiose	1.98	1.97	(52)
optical gotation	[4] _D -20.4	[N] 22 -18-4*	0.60(52)
036- M.P.	197-98	194 - 96°	(53)
	凶 ²² 48°	区 _D + 47°	(53)
	Repercentage Reylobiose in solvent(B) Optical rotation Optical	Scylobiose 1.98 in solvent(B) Optical [4] = 20.4 rotation 197-98 Optical [4] = 48	## 191° 192 = 93° ## 191° 192 = 93° ## 191° 192 = 93° ## 197° ## 198

III.25.9 Etamination of Praction VI and identification of

 R_0 , 0.81 in solvent (0), $R_{\rm Man}$, 0.62 and 0.80 in solvent (A) and (B) respectively. The sugar was crystallised from aqueous methanol, $\left[\checkmark \right]_{D}^{32} + 80.7^{6}$ (in water,C,1.0%). It was identify to be D-galactose by co-chrematography with an authentic sample.

III.25.10 Beamingtion of Fraction VII and identification of Demannosa.

 R_g , 0.12 in solvent (A) and R_g , 1.09 in solvent (C), $[<]_D^{30}$ + 12.9° (in water, C, 2.1%). The sugar was identify to be D_mannose by co-chromatography with an authentic sample.

REPERSICES

- 1. (a) Chopra, R.M.; Chopra, I.C.; and Nayer, S.L.,

 *Glossary of Indian Medicinal plants'; 172(1956).
 - (b) Chopra, I.C., Chopra, R.N. and Varna, S.S.;
 *Suppliment to Glossary of Indian Medicinal Plants's
 Page No. 72, (1969).
- 2. Howes, F.N., 'A Dictionary of useful and everyday plant and their common names', Page No. 18, First published (1974)
- 3. Fernandes, O., and Alfagess, C. Rev. Sanid. Lig. Pub.
 11, 525-35 (1936).
- 4. Caroline Sherman Lanford, Beatrice, Prinkelsteen, and Sherman, H.C., J Mutrition 21, 172-7 (1941).
- 5. De Moura, F.A., Compose (Univ. Sao: Paulo, Srasil). Brasil med. 61, No 20/21/22, 197-99 (1946).
- 6. Sadane, J.C., and Bashir Ahmad, J. Sci., and Research (India) 8B, No.2. 35-9 (1949) CA.43, 2708 h
- 7. Hazel E. Munsell, Louiso, Williams, Louise P. guild,
 Lucille, T. Kelley and Rober S Harris.; Food
 Research 15, 421-38 (1950) CE, CA. 45,3523 h.
- 8. Chung-Ching Su, Temching. Lu. and Yaw-Huli Lin (Natl. Taiwan Univ., Taipei). CHing Hao Hung Yeh Hisa. Hough Hai Chih (1-2) 33-4, (1963).
- 9. Chang-Ching Su, and Ta-Heiu Liao (Natl. Taiwan Univt.

 Taipei) Chung Mao Nung Yeh Maa Memch Mui Chih (1-2)

 42-3. of Preceding Mostr. (1964).
- 10. Agot, A. (Sta. Bosh. Aricoles, Canet, C.N.R. Joney-en-Joses, Fr.). Bull, Soc., Sci., Myg. Aliment (1968)

- 24 (1-3). 27-41 (Pa)
- 11. Knapp, Furn F., Nickolas, Harold. Phytochemistry, <u>8</u> (10), 2091-3 (1969) (Hng.)
- 12. Mai, G.A., Luh, B.S. Chung Ruo Hung Yeh Haa Hauch Hai Chih (Special Issue), 1-17 (Spg.) (1970)-
- 13. Shamtha, H.S., Siddappa, G.S., J. Food Sci., (1970), 35.
- 14. Maki, Mitsuaki., Sato, Yukio., Mossigaku Zasshi (1969) 22 (6), 406-10 (Japan).
- 15. Simmonds, N. W., Nature 173, 402-3 (1954).
- 16. Andrews R.S., and Pridham, J.B. Phytochemistry. (1), 13-18 (1967) (Eng.).
- 17. Bottier. R.A. Fr. I. 169, 727, Jan.5, 1959.
- 18. Bazarova, V.I., Sb. Tr.Lenningv. Inst. Sov. Torgovli. No. 23, 71-80 (1964) (Russ).
- 19. Suckley, S.H. (United fruit Co Norwood, Mars)., Phanolics
 Norm.Dis. Pruits Veg. Proc. Symp 4 th Norwood, Mars(1964)
 (1-6), discussion 16-18 (Pub.1965) (Eng.).
- 20. James F. Eheart and Branche S. Mason, J. Amer. Dist. Ass. 50 (2), 130-2. (1967) (3ng.)
- 21. James F.G. Hawkins, E.G. Jones, J.K.N. and Young G.T.; J.Cham.Soc. 390-4(1940) (Eng.).
- 22. Barrios, M.L. Ganzale, M.A. J. Ag. Univ. P.R. (1971), 55(2), 263-4 (1971) (Eng.)
- 23. Sanyal, A.K., Gupta, K.K., and Choudhary, N.K., Arch. Intern. Pharmacody. 149, (3-4) 393-400 (1964)(Eng.)
- 24. Bolivarde 1073. Claret Poses N., Am. M., Rechnologia, 12.
- 25. Perrell, Milliam J., Drouillard, Mark., Physiol. Chem. No. (1970).2 (2), 168-70(Eng.)

- 26. Lalla, 3,5, and Johas, D.S., Current Sci., (India).
 24, 92-3. (1955).
- 27. Lullar B.S. and Johar, D.S., Current Sci., (India).
- 28. Kri Korian, A.D., (State Univ. of New York, Stony Brook N.Y.)

 Been. Sot., 22 (4) 385-9 (Sng.) (1968).
- 29. Hady Lopes Boryes, Solutridady adstencia social (Hawana).
 46, 140-83 (1943).
- 30. Philip, L. Harris and Gev. Poland. Pood Research, 2, 311-19 (1937).
- 31. Grois bois, Micklele, Masliah P., Food Sci. Technol. Proc. Met. Congr. Int (1962) (Pub. 1969), 285-91 (Fr.).
- 32. Groshols, Michele, and Mazliak, P. (Sta. Proid, Ballevene).

 Paris) Fruits Paris 19 (2) 55-9(1964).
- 33. Pereira, J.R., Bustos, R.S. and Syngier, & Arch. Intelm.

 Pharmacology 144(1/2) 144-50 (1963) (Eng.)
- 34. Sanyal, A.K., Banerjee, C.R. and Das, P.K., Arch. Intern.
 Pharmacology, 155(1),244-48 (1965)(Eng.)
- 35. William S. Scott, Masel. M. Mckay, P.S. Schaffer and Thomas D. Fontaine. J. Chin. Invest. 28, 899-902(1949).
- 36(1) Vincenzo. Carelli, Paolo Marchini, and Aldo Tuccies
 Ann. Chein (Rome) 45, 1126-32 (1955).
- (ii) Vincenzo, Carelli, and Paolo Marchini, Ebid, 1133-45(1955),
- 37. Bartholomew Nagy, Vincent Modseleski and sister Marry, Marphy, T.J. Phytochem., 4 (6), 945-50 (1966) (Eng.).
- 38. Jain, Smeh.R . Planta Ned. 17(1) 98 (1966) (Eng.).
- 39. Knapp Furn F. Hickolas, Harold J. Steroids(1970), 16(3), 329-5 (Eng.)
- 40. Mapp. Pum J. Nickolas Barold Pum J. Nicko

- 59. Mikes, C... *Laboratory Hand-Book of Chromatographic Hathod *s, Est &d. Van Nostrand.P. 71 (1966).
- 60. Risvi, S.A.I., D. Phil. Thosis, University of Allahahad, (Endia) (1968.).
- 61. Andrews, P., Hough, L. and Jones, J.K.N., J.Am. Cham.
 Soc., 74, 4029 (1952).
- 62. Hamilton, J.K., Partlow, S.V. and Thompson, N.S., M. Chem. Soc., 215 (1950).
- 63. Aspinall, G.O., Rashbrook, R.B. and Kesslar, G.; J.Chesa. Soc., 215 (1958).
- 64. Meier, H.; Acta Chem. Scand.; 14 749 (1960).
- 65. Courtois, J.E.; Petek, F. and Kade. T.; Bull. Sec. chem. Bio., 40 , 2031 (1958).
- 66. Morgan, K. and O. Heil, A.N.; Cand. J. Chem. 37 ,1201, (1959).
- 67.(a) Tewai, 5.N., J. Anal. Chem. 176 604 (1960).
 - (b) Wilson, C.M., Anal. Chem. 31, 119 (1959).
- 68.(a) Marier, J.R. Soulet. MC, J. Dairy Sci.,42, 1390(1950).
 - (b) Dubois, M., Gilles, K.A., Hamilton, J.K. Rabers, PA., Smith, P., Anal. Chem., 28, 350 (1956).
- 69. Carego, A.S., J. Org. Chem. 30 , 924 (1965).
- 70.(a) Lederer, E. and Lederer, M.; 'Chromatographic Method's, 1st Ed. P. 88 (1966).
 - (b) Mikes, 0., *Laboratory Hand-book of Chromatography *, Elsvier's P. 166 (1955).
- 71. Trvelyan, W.E., Proctor, D.P. and Harrison, J.S., Nature

 166 , 444 (1930).
- 72. Ellis, G.P.; Cham. Ind., 902 (1966).
- 73. Belcher, R. and Godbart, A.L., 'Semi-micro-quantitative Organic analysis', 2nd Ed., 2. 164 (1954).

- 74. Barker, S.A., Foster, A.M., Siddigai, I.R. and Stacey, M., Talanta, 1 , 216 (1958).
- 75. Partridge, 4.4.; Stochem. J. 42 238 (1948).
- 76. Isbell, H.S.; and Frush, H.L.; Wethods in Carbohydrate
 Chemistry' (Ed. Whistler, R.L.) Academic Press, Inc.
 Vol. II, P. 117(1363).
- 77. 72.
- 78. Heeler, i., 'Methods in Carbohydrate Chemistry', (Sd. Whisther R.L.), Academic Press Ec. Vol. II, P.117, (1963).
- 79. Nisaki, A. and Smith, F., Agr. Food. Chem. 10, 104 (1962).
- 80. Fastuska, G.p J. Anol. Chem. 179, 427 (1961).
- 81. amith, F. and Montogomery, R., "The Chemistry of Plant Gums and Mucilage", Am. Chem. Soc., Monograph series, Reinhold Publishing Corporation, New York, P. 134 (1959).
- 82. Souveng, H.O., Kissling, H., Lindberg, Band MC-Kay, JE., Acta Chem. seand., <u>16</u> 616 (1962).
- 83. Tyminski, A. and Timell, T.S., J. Am Chem. Soc., 82, 2823(1960).
- 84. Garagg. P.J. and Lindberg. B.; Acta Chem.Scand., 14, 871 (1960).
- 85% Melchor, R. Fildes, J.E. and Nutten, A.J. Analyt. chem.
- 85.(b) Rafique, M.C. and Smith, F., J. Am. Chem. Soc., 76,2221(1954).
 (c) White, S.V. and Rao, P.S., J. Am. Chem. Soc., 75,2617(1953).
- 86. Chanda, 5.K., Hirst, E.L., Jones, J.K.M., Percival, E.G.V., 1289 (1950).
- 87. Cifchelli, J.A. and amith, P., Anal Chem. 25, 1132 (1954);
 Bid., 77, 1984 (1935).
- 88. Percival, E.G.V, and Willow, E.C., J.Chem.Soc., 1608 (1949).
- 89. Robertson, G.J. Spendie, T.H. J. Chem. Soc.,824 (1934).
- 90. Robinson, G.Les J. Chem. Soc., 330 (1934).

- 91. whistler, R.L. and Dugso, D.F., J. Am Cham. Sec., 74, 5140 (1952).
- 92. Haworth, W.W., Higst, E.L. and Plant, M.M.T. J. Cham. Soc., 1354 (1931).
- 93. Hirst, E.L. and Jones, J.K.M., J.Chem.Soc., 1278 (1948).
- 94. Whistler, R.L., 'Methods in Carbohydrate chemistry',
 Academic Press, Vol.V. P. 332 (1965).
- 95. andrews, 8., Hough, L. and Jones, J.K.N., J. Chem. Soc, 2744 (1952).
- 96. Bgown, F., Halsall, T.G., Hirst, E.L., and Jones, J.K.N., J.Chum. Soc 28 (1948).
- 97. Hough, L. and Powell, D.B., J. Chem. Soc, 16 (1960).
- 98. Aspinall, G.C., Rashbrook, R.B. and Ressler, G., J.Chem. Soc., 215 (1962).
- 99. Coldstein, I.J. and Whelen, W.I.; J. Chem. Soc., 170(1962).
- 100. James, J.K.N., and Painter, T.J.; J. Chem. Soc., 669, (1957).
- 101. Oyaw, M.O. and Timell, T.S., Canad.J. Chem., 38, 1957(1960).
- 102. Parila, C. and Bishop, C.T., Canad. J.Cham. 39 ,815 (1961).
- 103. Bailary, R.4.; "Oligosaccharides", International Segies of Monographs on pure and Applied Biology, Biochemistry Division, Vol. 4, Pergamon Pres, New York, P, 51(1965).
- 104. Handerson, M.E., Hough, L. and Fainter, T.J., S.Chem. Soc., 2519 (1958).
- 105. Whistler, R.L. and Durso, D.F. J. Am. Chem. Soc. . 73.4189 (1958).
- 106. Boismive, Francis, R.C., Straichen berger, Gilles, F.R. Lachat, Paul R.M., Gar. Fr. Appl. (1968).

CHAPTER - IV

CHEMICAL EXAMINATION OF THO PLAYOLOGOS

AND AN AUTHOCYANIN FROM THE PRUITES OF

GARDENIA GUENIFERA LIBIN.

IV.) In the present chapter chemical examination of two flavonoids and an anthogyanin from the fruits of <u>Gardenia</u> garmifers <u>Linn</u>. has been described.

Gardenia gumnifera Linn.. commonly known as 'Dika mali'. belongs to the family Rubiacese', is a small unarmed nearly glabrous shrub with resinous buds. Leaves, sessile or subsecutie. Us - Us inches in long, obovate, acute or abtuse, shining, base obture, acute or cordate sometimes puberulous beleath. Stipules, connate, truncate or mucronate. Flowers, 1 - 3 together, subsecutie, calyx, pubesent, lobes short, ovate, acute. Corolla, white turning to yellow, its tube 1-2 inches long, glabrous or pubesent; limb 1-3 inches, across; lobes 5 ablong, abtuse. Fruit 1 - 15 inches in long, ellipsed or oblong and smooth pericarp thin, placenta, 4 - 5. Plowers during March and April.

The plant is found in Sandelkhand region and its distribution in Southwards from chota Nagpur and Sombay. It is tropical and subtropical shrub, cultivated, Ornamentally.

together with a similar substance yielded by G.lucida. Gun of G. gumnifera is antispasmodic, Carminotive, antiseptic, stimulant, anthelmintic. It is also used in veterinary medicine to keep off flics from sores. Its ernamentally flowers are often perfused. Some species used in local dyeing ardenia seeds are useful in enhance healing of soft tissue.

TV_2 The details of recentch work reported in the likerature on this plant is given in the next page.

		peto	Constituents	Partes I	le ferances
1.	Garden la	***	Stable yellow food colouring agent	•	(1979) ⁵
2.	Garden La	40	Colour of natureal dye (Capa Jasmine dye)	Pruits	(1976) ⁶
3.	Garden Le	•	Food colouring agent (Yellow, green & blue pigments)	Seeds	(1978)7
4.	Garden ia	1600	Setraction of Grange. yellow pigment from defatted gardenia	· ·	(1975)8
5.	Carden la	Florida	Mann itol	Poliage leaves fruits	6
6.	Garden la	Florida (grandi flora)	Crocin & crocet in (colouring matter of safran group)	Fruite	(1922)10
7.	Garden ia	Grandi- flowa (Japanes wongshy)		Saads	(1944) ¹¹
8.	Gardenia	Jasminoi es and grandifl	d 10-acetyl geniposi a picrocinic acid	Cerruits	(1976) ¹²
9	• Garóania	Jasaindi des 4 gr diflora	Three new glucoside tem Gardenoside, shanshisidem Me deum cetyl asperulosim date, geniposide, geniposide, side	Fruits	(1974) ³³⁸
10,	• Gardenia	Jamaino idea égi diflora	Tarchnoside, tarenna ra gracilipes, gardena side, geniposide, & geniposidio acid	o Cells tissue	ed (1981) ¹⁴
11	. Gardenia	Jagmine- ides	. Cleaphilic natural dye (lipophilic dye for foods cosmetics	.	(1979) ¹⁵
12	. Gardenia	Jamino 18es	. Crocin glucoside		(1950) ¹⁽
13	. Gardenia		m Gardenoside(8,10-di hydrologanic acid) A scandoside-Ne est		(1974) ¹⁷

(Continued)				
Genus :	ipecles	Constituents Pe		eforances
The state of the control of the state of the	ides 1	eniposide & genipin - P-P-gentiobioside (2-new iridoid glu-	Praiks	(1973)
15. Cardenia	ides 4	Gardenosida, genipa- side (Two new iridoid glucosida)	Fruito	(1969) ¹⁹
16.Garden La	Jasmino- ides	Crocin extraction	Faits	(1974) ²⁰
17.Gardenia		Shanzhiside, (a new iridoid glucoside)	Fruits	(1970) ²¹
18.Garden La	1300	Genepin-l-7 -genti- opioside(New iridoid glycoside)	Pruits	(1970) ²³
19.Garden La		Monacosane, P -sitost- erol, D-Mannitol.	Fraits	(1964) ²³
20. Garden ia	Lacida (Dikamali cum)	Hestaccayl p-counts- te(a new phonolic ester)	Leaf bud prudata	(1980)24
21. Gardenia gam	Dikamali gum	5.7.3 ⁸ .4,-tetrahydro- xy 6.8 -dimethoxy flavone	Guns	(1977) ²⁵
22. Gardenia gum (Dikamali gum)	60	Gardenia, Demethyl tangerstim, nevadensin 5,7, dihydroxy. 6,3,4,5 tetra methoxy flavome		(1971) 36
23. Garden ia gum(Di)ta	tạc ida	Gardenin A.S.C.D & S	Resinens exudate	(1970) ²⁷
mali gum) 24. Garden ia gum	•	a mix. of p-commasic esters of higher alcohals(C22 C26)	\$10	(1979) ²⁸
25.Garden is	Liac Ada	D-Mann it ol	Rook. Dark	(1966) ²⁹
26.Garden is		Cleanonic aldehyde, erythrodiol, 19- < - hydroxy crythrodiol- sitosterol, D- munitol.	Stem bark	(1977) ³⁰

	Spectos	Congt ituants	Perta	
7. Ogrden in		D-Mannikol, hetaacetyl- D-mannikol, D-mannikol- hetabens Oate, gardenin A,SES, oleanotic acid, <-Myrin and B -sito- sterol.	Roots	(1979) ⁵¹
28.Garden ia	Turgida	D-Mannikol,	scudat-	(1925) ³²
29 . Garden is	Turg ide	f-sitoserol,D-mannitol, oleanolic acid methyl aster, gypsogenic acid methyl ester, & hedera- genic methyl ester	wood &	(1973)33
30. Gerden ia	Forbergii	Three new flavones	aud estudate	(1979)34
31.Gardenia (African varieti =	Pomodora & vogalli	D-mann itol	400	(1975) ³⁵
es) 32. Garden ie	talifolis	Mannitol & sitosterol.	Grido	(1969)36
33. Garden La		Demannitel, sitestarel deanolic, siaresinolic, spinosic acids and haderagenin	sten ly-azk	(1975) ³⁷
34. Garden La	Latifolia	3-episia resinolic acid (andw triturpene acid)	Bark	(1975) ³⁸
(Agrican	t omant Li	D. Mann it ol	Root- bark	(1974)39
varieti- es) 36.Gardenia		Crocin. Crocetia	600	(1954)40
37 . Garden te		Design	400	(1976)41
36. Garden Le		Pharmacology	Apots 6	£ (1936) ⁴²

jel

root, leaves, stem, bud and fruits, gum of genus have been extensively examined for various plant products. Since no study on anthocyanin and flavomoids compounds from the fruits of Gardenia gumnifers, therefore, it is worthwhile to investigate thoroughly the plant fruits of G. Gumnifers for their chemical constituents.

IV.1 EXTRACTION AND ISCLATION OF PLAVONOIDES AND ANTHOCYAN DE PROJETA OF GARDENIA GUELPERA

The fruits of Gardenia gumnifera were purchased locally and identified for their authenticity in the Botany department of D.V. Postgraduate College, ORAL, (Bundelkhand university).

Petrolium ether (60-80°) in a suchlet extractor. The defatted material was extracted with ethanol (95%) on a steam-bath in several lots. The total extract was concentrated at reduced pressure to a reduish brown viscous mass. It was refluxed with petroleum ether (60-80°) to remove the fatty material and resulting residue, still viscous mass, was poured into 1 litre of distilled water with vigorous stirring. The water soluble and insoluble fractions were separated by separating funnel and successively subjected to liqued-liqued extraction, using petroleum ether, bensene, sthyl acutate and acutome respectively.

The benzene fraction of water insoluble part was

iel

evaporated whereupon a light-yellow substance was obtained. The purity of the substance was tested on TLC which give a single spot. This pal yellow mass on crystallisation from acetone mathanol (1:1), gave an yellow compound, D, m.p.349°. The ethyl acetate extract of water soluble part was subjected to column chrometography over a silica gel G. The benzenesthyl acetate (1:9) cluste of the column yielded a dark yellow

coloured compound (8), having m.p. 1500.

shaken with solvent other in a separating funnel, several

refluxed with seatons (8.0.%.) in different lots. The whole setract was reduced to very small (20 ml) volume wheroupon a solid mass (Orange-red) was obtained. The acetons acetate part gave a simple spot on TLC using ethyl acetate-acetons (9:1) and 8...4 (4:1:5 v/v) systems, showed a single compound in the extract. The compound (F) was crystallised from methanul having m.p. 300

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With according concentrated, aubjected for Tring Thomas extraction with athylecotors and abod , cay deal. SOURCE CONCERNION Water soluble fraction 9000 Poured into large oxchess a silkes gol & elimits giltrate. Defatted with Petroleum ether(60-80°) subjected to colourn chromatography over of discillation unter acatate(199) was abitlecetate estrect Aller Constant 90 Roglumes with etherol collected as in a southlet for 26 hours. Total Control Concentrated, treated with 301GH CAROLINA COLORODO Put. ather and filtered Water inschible fraction concentrated and subjected to laquedlighed extraction with Stop (Cut) b Det Fact Residen (Fatty Battero) solether(Solvent ether) (Patty matters) shoken with Campone extract concentrated Therest layer

SECTION - A

IV.S CHEMICAL STUDY OF COMPOUND (D)

ethanolic entract of water insoluble fraction of ethanolic entract of fruits of <u>Gardenia cummifera</u> afforded a compound (D), m.p. 349° and molecular formula, C₁₅H₁₀O₅°. It was isolated from the fruits of Gardenia gummifera as described on page |3| and was shown to be single entity by paper chromatography and responded to the following colour reactions:

- (1) It gave pink colour in Shinoda reduction 43, but did not give pink colour with hydroghloric acid alone.
- (11) It gave intense yellow colour with characteristic fluorescene by conc. sulphuric acid.
- (111) It gave an yellow orange colour with ethanolic ferric chloride 46.
- (iv) It produced yellow colour with liqued amagnia which showed yellow fluorescene in UV light 47 .
- (v) A yellowish colour was obtained on treatment with sodium hydroxide solution, which was stable on heating 48 .
- (vi) No change in colour was observed on addition of vanillin hydrochloric acid reagent to the compound (D).

The above reactions suggest that the compound (D) is a flavone derivative possessing following skeleton.

It gave negative Molisch's test indicating thereby the aglycome nature of the compound.

The presence of this skeleton of pale yellow coloured compound (D) also supported by the absorption maxima of the compound at 269 nm and 336 nm. The skeleton accounts only for $c_{15}^{\rm H}_{10}^{\rm O}_2$, which suggests that the remaining three caygen items may be present as three hydroxyl groups in the aglycone. The compound formed a trimsthyl ether and triacetate on methylation and acetylation respectively, confirming the presence of three hydroxyl groups in it. Thus the compound may be represented as below a figure of the compound of

The relative position of these three hydroxy groups have been assigned on the basis of various colour reactions, degradation and spectral studies of the compound.

The compound on oxidation with neutral potassium permanganate gave a compound identified as p-hydroxybensoic a cid.

This reaction shows that one hydroxyl group compound (D)

is present at position -4' of B ring of the compound. This was further confirmed by the following facts:

- the solution (Shinoda reduction) of the compound a blue colour 49.50, was obtained, showing the presence of free hydroxyl group at position ~4.
- (ii) in ethanolic compound showed a bathschromic shift of 40 nm of Band 1 (from 336 to 376 nm) by the addition of fused sodium acetate, indicating the presence of hydroxy group at position 4' or i^{51} . The positionity of hydroxyl group at position -3 was eliminated by the fact that the yellow colour given by the compound with aqueous sodium hydroxide was stable on heating 48 .
- (111) A bethochrosic shift of 56 nm of band 1 (from 336 nm to 392 nm) without a decrease in relative intensity was cheered by the addition of sodium ethoxide to the ethanolic solution of the compound. This shift is diagnostic 53,54 for the presence of free hydroxyl group at position =4.
- (iv) A single well defined peak (269) of band II of the compound in ethymol also confirmed the presence of 4 \sim substituent in the B-ring 52 .

The compound (D) on fusion with potassium hydroxide gave 55 a compound identified to be phloroglucinol. This degradation showed the presence of free hydroxyl group at positions -5 and 7.

Compound (D) S% Aqueous Potassius hydroxide

shloroglucinol.

the presence of free hydroxyl group at position -5 was further confirmed by the following facts:

- (i) The compound (D) gave an orange-red colour with Dimroth's respent (scatyl pyroborate) 56.
- (ii) The compound gave bright yellow colour with mathemolic singular time about the presence of free hydroxyl group at position =5⁵⁷. The colour did not change on addition of citric acid showing the absence of hydroxyl group at position =3 in the molecule ⁵⁷.
- (111) when the compound (n) in acctone was traited with a solution of horic acid and citric acid in acctone, it give a yellow colour with yellowish green fluorescence. This shows the presence of methodyl or hydroxyl group at position -5.28.
- (iv) An ethanolic solution of the compound gave green colour with the ethanolic Perric chloride 46 .
- (w) Bathochromic shifts of 46 nm in Band I (from 336 nm to 382 nm) and of 9 nm in Band II (from 269 nm to 278 nm) were cheerwed by the addition of a few drops of ethanolic aluminium chloride to the ethanolic solution of the compound. This showed a free hydroxyl group at position ~5 of the aglycone \$9,60.

The presence of free hydracyl group at position -7 of the compound (T) was supported by the floklowing facts :

- (i) Fink colour was given by the aglycone with vanillia hydrochloride respent, indicating the presence of free 5,7, -di-
- (11) A buthochromic shift of 9 nm of Band II (from 269 nm to 278 nm) was observed on addition of a little fused sodium southte to the shimmalic solution of the compound, confirming the

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presence of free hydroxyl group at position "7⁵¹. The compound also did not give any precipitate with neutral lead and acetate showing the absence of Ortho-dihydroxy grouping.

Hence, on the basis of observations, the compound (D)
has been assigned the following structure 4. 5. 7 -trihydroxy
flavone (A pigenin).

IV. A REPERIMENTAL

and insoluble in petroleum ether, benzene and water. It gave all positive tests, characteristics of flavonoids, as described on page 133.

TV.7 CHROACTOOR PIX OF THE COMPONED

The parity of the compound was checked on whatman NO.1 filter paper when a single spot was observed in each case using following solvent system :

	-	400)	n- But, an	ol-seek ic	acid-water	(41213	v/v)	0.37
1	1	4	1	/hanol	esturated	with water				0.96
						ac 10-water(4/4)	0.00

IV. B GILLOW T. L N. LES IS G THE COMPARED

Pand			Calculated for	C15 10 5
C	66.62		66.60%	
M o	4.0%		3.70%	

. Ág

IV.9 ACCITATION OF THE COMPOUND

The compound (50 mg) was acatylated with acatic anhydride (50 ml) and pyridine (3.0 ml). The reaction minture was left overnight and poured in ice-cold water with constant stirring. It was filtered, washed will with water, dried and recrystallised from methanol to yield acatyl derivative, m.p. 187 - 88°.

IV.10 DETERMINATION OF ACETYL PERCENTAGE

The acetyl percentage in the acetylated derivative was determined by the method of wisenberger 63 as described by Godbert and malcher 64 .

Found

Calculated for C, H, O, (COCH)

Acetyl group a 31.95%

32.57 %

IV. 11 ASTHEL TICK OF THE COMPOUND

The compound (40 mg) was taken in dry acetone (20 ml) and was mathylate with dimethyl sulphate (5 ml) and anhydrous potassium carbonate (1.0g) by reflucing it on a water-both for 24 hours. The reaction mixture was cooled, filtered and poured over crushed ice, whereupon a yellow mass was settled to make it is a diltered, washed and recystallized from ethanol, map. 158°.

IV.12 DETER THATION OF METHOCEL GROUP PERCONTAGE

The methodyl percentage in the methylated derivative of compound was determined by the method of Belcher, Fildes and Nutten 65 .

Found

Calculated for C₁₅H₇O₃(OCH 3)₃

Methodyl group = 18468%

IV-13 POTASSIUM PERMANGANATS OXIDATION OF THE METHYL STREET

The methylated compound (20 mg) was exidised with neutral potassium permanganate solution under reflux for 6 hours. The reaction mixuture was cooled and the excess of manganese discide was destroyed by adding sodium bisulphate to it. The resulting solution was acidifed with dilute hydrochloric acid, whereupon a white compound was separated out. It was filtered and crystallised from ethanol, m.p. 178°. It was identified to be anidic acid by its mixed melting point and co-chromatography with an authentic sample. (Rg 0.37 in m-butanol saturated with ammonias apray bromophenol blue solution).

IV-14 UV AND VISIBLE SEATEN OF THE COMPOUND

UV and visible spectra were recorded on Beckman Model DV spectrophotometer.

50)		en energia esperia de la composição de la c La composição de la compo	5	n.R	
(1)	Compound+Sthanel	269,336	(65)		
(11)	Compound +Sthano), Wall of	278,386	9,	40	
(111)	compound+Sthanol+Alcl3	278,382	9.	46	
(1v)	Compound+Sth and Anda	277,392	8,	\$6	

LA SPACIALM OF COMPOUND (D)

Following prominent peaks (cm^{-1}) were observed in the LR spactrum of the compound :

3442, 3289, 1660, 1625, 1590, 1580, 1355, **1205**

SECTION B

IV.15 CHEPECAL STUDY OF THE COMPOUND (2)

from the ethanolic extract of <u>Gardenia guantiera Linn</u> as described on page |3|. The molecular formula was found to be $C_{36}^{H} C_{26}^{O} C_{11}^{O}$. It had R_{g} value 0.41 in b-Butanol: Arctic acids water (4:1:5 v/v) system. On reflucing with 7% ethanolic sulphuric acid it gave a water insoluble aglycome, having molecular formula $C_{18}^{H} C_{16}^{O} C_{1}^{O}$. The compound responded to a positive Molish test showing that the presence of a glycside. The extract nature of the glycoside was confirmed by the identification of and chracterisation of the aglycone and sugar molecy database on acid hydrolysis of the compound.

IV.16 STATE OF THE ASLECTIA

An yellow aglycone, $c_{18}^{H}_{16}^{Q}_{\gamma}$ had nop. 204°. Ethanolic solution of the aglycone gave the following colour reactions and chamical tests.

- (i) Various shades of red colour were obtained when it was treated with.
 - (a) 19 + Hel 43
 - (b) Zine + Hel 66
 - (c) Na/Hg + Hcl 45
- (11) It produced a dull green colour on treatment with ethanolic ferric chloride.

These colour reactions suggest that the compound may be a derivative of flavone or flavanone.

(111) then it expected to vapours of amonda liqued on a

filter paper, the compound turned to darkyellow colour and showed fluorescence under ${\rm UV}^{47}$ light.

- (iv) A dark yellow brown colour was obtained on treatment with aquous sodium hydroxide.
- (v) It did not respond to a positive reaction with 2,4- Disatro Phenyl hydrazine reagent 67.
- (vi) It could not be reduced with Dodium Borohydride showing the absence of flavanone skeleton 68 .

From the above reaction it is obvious that this compound is a flavone derivative and should have the following skeleton.

- (vii) It showed a positive reaction with ethanolic Borie acid and sodium acetate 69.
- (viii) It responded to a positive reaction with Zirconium exychloride in presence of citric acid 57 .

These reactions indicates that there is a hydroxy group at position =3. Thus the skeleton of the compound is designed as 3-hydroxy flavone or flavanol having the following skeleton.

cayges items, two in the bensopyrone nucleous and one as OH at positive -3 have been accounted for the compound(S).
The compound (E) was found to contain three methodyl groups
by Ziesel's method, IR peaks at 2850 on and 1185 cm 171,72
and formed a discetate showing the presence of two free
hydroxyl groups in its structure.

Thus the aglycone can be represented as, and this accounts for all the seven oxygen atoms in it.

The relative position of methodyl groups and of the hydroxyl group have been determined by the degradation studies of the compound and its colour reactions.

when compound was exidised with neutral potassium pernanganate, veratric acid was one of the products isolated from the exidation mixture.

glycone of the compound pormandana

Veratric acid

Presence of this compound clearly indicates the position of two methody group at -3'and - 4' positions. Thus the structure may be represented.

The aglycone of compound (I) gave a green colour with ethanolic ferric chloride 46 and responded to a positive test with ethanolic borie acid in presence of citric acid 47,69 , showing the presence of a free hydroxy group at position -5. Thus the structure may be written as follows s-

The possible position for the remaining methodyl group appears to be - 7 position in the ring A, which is indicated by the absence of a positive colour reaction with vamillin hydrochloric scie⁶¹ reagent. Thus the structure of the compound as follows :

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this structure of the aplycone is supported by the following colour reactions and spectral properties of the compound.

(1) The presence of -7, 4' - dimethoxy flavanol skeleton 73
is shown by peak at 1604 cm⁻¹ in the IR spectrum of the compound

- shown by the fact that the compound did not shown any bathometersic salift (max. 255 nm) in the UV region on addition of fact sodium acetate to it ethanolic solution.
- (111) The presence of free hydroxyl group in position -5 has been shown by the following facts :
 - (a) has aglycone of the compound was treated with ethanolic alcl, on a filter paper, it produced fluorescence under J.V. light 77.
- (iv) The presence of free hydroxyl group at position -3 has been shown by the fact that a bathochronic shift of 46 am (mx. changed from 360 nm to 415 nm) was observed on addition of the compound 76.
 - (v) The absence of free hydronyl group at -3 and -4 position is confirmed by the fact that the compound did not show any bathochromic shift (cast 369 nm) in visible reagion on each team of North less and modern contate.

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IV. 17 DESTIPICATION OF THE SUGAR

compound (E), was found to respond to positive Molish's test and reduced Febling solution. It gave a spot with R value 0.35 in n-Bitanol sacetic acid: Mater(4:1:5 v/v) system and formed an ossione, having m.p. 190 with Phenyl hydraxine reagent, suggesting the sugar to be rhammone. This was confirmed by its co-chromatography with an authentic sample.

TV. 18 STEDY OF GRIGINAL COMPEND (E) AND POSITION OF

From the above discussion it is obvious that compound(3) in a charmoside which can be represented as below :

since there are free hydroxyl groups at position -3 and -5 hence it is clear that sugar residue could be linked in either of these two positions. (-) or -5 positions.) A clue to the position of the sugar linkage has been obtained by comparing the properties of the glycoside with that of the aglycome.

It has been sentained earlier that the aglycome responded to a positive reaction with sodium borate and also Sirconium to a positive reaction with sodium borate and also Sirconium to a positive reaction with sodium borate and also Sirconium to a positive acid showing the presence of charles acid showing the presence of charles acid showing the presence of the pres

Z661

condition, Throuside did not respond to these tests from which it appears that sugar is linked at the position -3 of the aglycone residue.

on methylation of the compound (2) with distomsthane and subsequent hydrolysis of the methylated derivative, a compound was obtained which was identified to be quareetin 7.5, 3, 4 -tetra methyl ether, m.p. 1920 80 and the spectral properties (max. at 253 nm and 362 nm) 75,79. This confirm the attrachment of the sugar at position-3 of the aglycome.

The glycoside did not reduce Fehling's solution nor gave a positive test with aniline hydrogen phthalate, reagent, suggesting that aldehyde group of the sugar is not fee 79 and is involved in glycosidic linkage.

The glycoside on periodate oxidation consumed 2:1 makes of particulate and produced 1:2 makes of formic acid per make of the compound, showing that the sugar was present in the pyranose form. The glycoside got hydrolysed with emulsin showing that the sugar was linked with the aglycone through the A —1 inkage $\frac{96}{2}$.

The glycoside (E) thus can be represented as follows :

POLINICAL COL

This structure of the compound(E) clearly explains the following spectral correlations :

- (1) The compound was found to be stable in 0.002H sedium ethylate showing the bathochromic shift of 12 nm (max. changed from 357 nm to 369 nm) in the visible region of spectrum, showing the absence of free hydroxyl group at positions ~3 and ~6'81.
- (2) The presence of peak between 835 cm $^{-1}$ to 810 cm $^{-1}$ in the I.R. spectrum of the compound is in confirmation with the pyranose structure of the sugar 61,82,83 .

Z661

ZGEL

TO PRINCIPAL

IV.19 IN CLATICE AND PURISICATION

An yellowish brown coloured compound (3), was isolated and purified as described on page 13/ . It was recrystallised from chloroform-methanol(9:1) mixture, m.p. 1900.

IV.19.1 HOMOGENETTY OF COMPOSED (E)

The homogeneity of the compound was checked by paper chromstography on Whatman No.1 filter paper using following solvent systems :

- (i) n-Sukanol-acetic acid-water (4:1:5 v/v)
- (11) Acotic acid-conc Hel-Water (30:3:10 V/V)

Spot was developed by exposing to the vapours of assonia. A yellow single spot was deserved which showed fluorescence under U.V.light.

SLEMESTAL MALES IS

			Calculated for C24H26 11								
Pot	m d		******								
2.00	LIGHT CHILDREN			C	33	58.6	196				
C		57.8%		11	89	5.3	%				
И		4.8 %									

IV. 20 HOD ROLYS IS UP THE COMPOUND

The compound (300 mg) were dissolved in minimum quantity of ethanol and in 100 ml round bottom flask. (50ml) of the 7% sthemolic sulphuric acid were added and the seaction mixture was refluted on a water bath for 10 hours.

The hydrolysed was cooled, solvent distilled off, diluted with water and filtered. The precipitate was dried in Yechen extend the store services chloroform (5:5 v/v) and thanky cocycetties from members to yield a yellow coloures

CPUNCEIPIEDED

ZEEL

N.21 DESTETICATION OF SUGAR

The eyrup detained after the hydrolysis of the glycosids was estamined paper chromatographically using n-Sutanol-acotic acid-water (4:1:5 v/v) solvent system. The developed chromatogram was airorind, sprayed with aniline hydrogen phthate and on heating at 120° for 10 minutes, one spot, R_g value 0.35 in n-Butanol - acetic acid-water(4:1:5 v/v) system was observed which corresponded to rhamonose. This was further confirmed by co-chromatography with an authentic sample.

N. 22 EXAMINATION OF THE AGING GIE

M.P. 2050, soluble in other, acctome, ethanol and methanol, sparingly soluble in chloroform but insoluble in petrolemm ether, benzens and carbon tetrachloride.

HOMOGREETTY OF THE AGLYCLICAR

The pair ity of the eglycone was cheeked by paper chromatography on Whatman No.1 filter paper using n-Butanol-acetic acidwater (4:1:5 v/v) and phenol saturated with water in each case a single spot was observed.

SLEMSTAL MAINS IS

	Lone		parasin	Reference or								4	N	9,	19		
	0.0	Å,											6	2.	076		
C	, «	A				174							4		57	6	
1	4 .	M		4	.5	16	100	-		al se							

Z661

IV. 23 ACSTYLATION OF THE AGINCONE

The aglycome was acetylated using acetic anhydride and pyridine by the usual method of acetylation. The acetyl derivative was crystallised from acetone, m.p. 180°.

IV - 24 DETERMENATION OF ACETYL PARCENTAGE

The percentage of the acetyl groups in the acetylated product was determined by the method of Wiesenberger 63 as described by Belcher and Godpert 64

Posts

Calculated for Class m 20.6%

Acetyl percentage = 20.1%

IV.25 PRETERLATION OF AGLYCONS

The aglycone (4 0 mg) was taken in dry acetone (20 ml) and was mathylated with dimethyl sulphate (5 ml) and enhydrous potassium earbonate (1.09) as usual method as described on page 138-

DETERMINATION OF METHORY'S GROUP PERCENTAGE

The determination of methodyl group in the aglycome was done by Riesil's method as described by Halcher Fieds and Nutten.

FOUNA

Calculated for C15H, O4(OCH3)3

methoxy groups

methoscyl groups

a 26.3%

a 27.03%

IV.26 POTAS TUM PERMANGANATE OMBATION OF THE AGINCONE

The aglycone (20 mg) were treated with aqueous potage ium permanganate under reflux for four hours. The reaction mixture was enoled and excess of manganese dioxide destroyed by adding medben stadionics to be a passition of hydrochloric acts a

a white precipitate was separated out which was crystallised from acatons, m.p. 120 -21°. It was identified to be veratric acid, Lit. 84 m.p. 120 -22°.

IV.27 METHYLATION OF COM-OUND (2)

The Glycoside (25 mg) was methylated by using (50 ml) ether solvent of diasomethane by the usual method. The methylated derivative was crystallised from chloroform having m.p.168-70°.

IV.28 PERICDATE OXIDATION OF THE COMPOUND (E)

The glycoside (20mg) dissolved in 25 ml ethanol and 25 ml distilled water were treated with 25 ml of .1M sodium metaperiodate solution. A blank was also prepared similarly.

The periodate oxidation was estimated by titrimetric method of Jones stal 35 15 ml. aliquote of the solution was taken out from the reaction mixture.

Molecular weight of the compound	500	2190
Por 15 ml aliquote of the solution O.1N sodium hydroxide consumed		0.35 ml
0.1N Hypo consumed		0.9 al
For each mole of the glycoside moles of periodate consumed	-633	2.1
moles of formic acid liberated	410	1.2

IV.29 SYSCIRAL STUDIAS

All the spectral measurements were taken using medium model Due spectrophotometer and absolute ethanol as the solvent system.

Solven	t and Reagent	max n m	siet n n
(A) GL	yeosida (E)		
(1)	Thanol	268, 357	
(11)	Sthanol/Alela	380	- 23
(111)	Sthanol/NaOst	369	- 12
(iv)	Sthanol/Na OAC	270	2
(A)	Sthanol/Borie acid + Na GAC	361	•
(B) Aq	lycong		
(1)	Sthanol	255,369	
(11)	Shanol/Alel3	415	- 46
(111)	Ethanol/Ra OAC	255	
(iv)	E thanol/Boric acid + Na OAC	368	400
(c) Aq	lycone of mathylated gly	cosida	
(1)	#hanol	253,362	•
(11)	Sthanol/Alcl3	421	59 -
(111)	isthanol/Na Ovc	252 -	
(IV)	Ethanol/Naoet	260,403	- 41

IV. 30 I.R. SPACTRUM

The prominent peaks in the I.R. spectrum of the glycoside are at 3350 cm⁻¹, 2850 cm⁻¹, 1604 cm⁻¹, 1500 cm⁻¹, 1450 cm⁻¹, 1185 cm⁻¹, 1150 cm⁻¹, 1130 cm⁻¹, and 835 -810 cm⁻¹.

SECTION ... C

IV. 31 CHEMICAL STUDY OF THE COMPOUND (P)

An Grangish-red compound (F), m.p. 300°C was isolated from the acetone extract of water soluble fraction of <u>Gardenia</u> gumnifera <u>Linn</u>, as described on page 131. The compound having molecular formula C₂₁H₂₁Q₁₀, was shown to be a single entry by paper chromatography.

The ethanolic solution of the compound gave following reactions :

- (i) It gave positive Molisch's test showing the presence of sugar moisty in the molecule.
 - (ii) In did not reduce Fehling's solution.
- (iii) It also did not respond to the positive test with aniline hydrogen phthalate 45 .

The above reaction ((ii) and (iii) specific for free aldehyde group in the sugars), suggesting that reducing group of the sugar moiety is not freely involved in linking end.

when the ethanolic solution of the compound (F) was heated for 5 minutes on boiling water-bath and it was extracted with amylalcohal, produced a violet colour with aqueous sodium acetate, showing it to be an anthocyanidin, therefore the original Compound(F) shall be an anthocyanin. On acid hydrolysis of glycoside(F) an aglycome and sugar moiety was obtained.

IV 33 AGIACGIV

The F values of the adjycone ware found 0.71 and 0.52 in scale acids water (30:3:10 v/v)

system and (5:1:5 v/v) system respectively. The aglycone of the compound (F), gave bluish red colour of the corresponding coloured base, with the treatment of sodium acetate and sodium carbonate. This colour change from bluish red to red was found quite stable in sodium acetate, solution. The stability in coloured base is well known in the case of flavylium salts having free hydroxyl groups at -3, and -5 positions. The aglycone did not respond positive Ferric chloride test, indicating, thereby the absence of any catechol unit in the molecule.

chloric acid solution was found to be 530 nm and in methanolic hydrochloric acid 520 nm. According to Harborne this shift from solvent to solvent corresponds to the palargonidin. But the compound (F), gave λ max 513 nm in methanolic hydrochloric acid. In case of anthocyanins it is generally known that substitution of one hydroxyl group lowers the λ max by 5 nm in the aglycone did not show any bethochromic shift of the absorption maxima in the visible region even with aluminium chloride. This confirms the absence of any catechol unit in the molecule 57.

From the foregoing discussion it appears that the compound (F) is a palargonidin derivative.

IV.33 DENTE FCATION OF SUGAR

The sugar was identified by paper chromatography in n-Sutanols acetic acid: wa-ter (4:1:5 v/v) system, which revealed a single spot with Rf value 0.18. It formed an osazone with the treatment of Phenyl hydrazine as usual method. This suggests the presence of glucose. It was further continued by co-chromatography with an

authentic sample.

From the above evidence it is clear that the compound(F) is a pelargonidin derivative with glucose unit attached as sugar moiety in the glycoside linkage and can be represented as follows:

GLYCORDIC IV.M POSITICA OF

The position of glycosidic linkage in the glycoside was determined on the basis of distinct absorption maxima in the visible region. The addition of sugar residue to the 3-position of the anthocyanidin molecule causes a large colour shift towards shorter wavelengths, but further addition of sugar to the 5position has little effect on λ max so that there is very little difference in colour between 3-glycoside and 3,5-diglycoside. Pelargonidia 3-glycoside has λ max 507 nm in methanolic hydrochlorie acid and its 3,5-diglucoside has λ max 504 nm. But the pre ent glycoside has A max, 513 mm which indicates that sugar molety is attached at -5 position 52.

It is further confirmed on the basis of biogenetic considerations and negative colour test with vanillin hydrochloric acid reagent 69. Gran compound (F) was subjected to neutral potassium permanganate exidation, p-hydrosy bensoic acid was obtained. This confirmed the structure of the side phenyl ring (8) of the molecule compound (P) Kmnou

p- hydroxy bengoic

that sugar is present as monosaccharide with the consumption of two moles of periodate with the liberation of one mole of formic acid per mole of the glycoside, from which it may be concluded that the glucose is present in the pyranose form. The pyranose structure of the sugar is also confirmed by the presence of medium peak in the region of 845 - 820^{CM} in I:R. spectrum of the compound 73,74,87.

The Glycoside was completely hydrolysed with the emulsin, which is specific for β -linkage. Hence, the nature of linkage between glucose and aglycone moisty seems to be β -linkage.

The above all evidences, suggest that the compound (F), is Pelargonidin 5- β -glucopyranoside and may be represented as below:

Pelargonidin = $5-\beta$ -glucopyranoside Compound (F)

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V.35 DIGLATION AND PURIFICATION

The compound (F), m.p. 300°C, was isolated from the fruits

f Gardenia cumnifers Linn, as described on page (3). The compound
has repeatedly crystallised from methanol, till a pure product was
btained.

V. 36 HOMOGENIETY OF THE COMPOUND

The homogenisty of the compound was checked by paper chromatography on Whatman No.1 filter paper using following solvent systems:

- (1) n-Butanol: Acetic acid: Water (4:1:5 v/v)
- (11) Acetic acid sconcentrated hydrochloric acidswater(30:3:10V/
- (111) Acetic acids concentrated hydrochloric acids Mater(5:1:5 V/V

In each case a single spot was observed.

elemental analysis

Po				Calcula	ed)	for c ²¹ 21 c ¹⁰
C	400	54.52×		C		53.8%
М	#3	3.95 %			100	4.48%

The glycoside (50 mg) was dissolved in small quantity of 5% ethanolic hydrochloric acid(50 ml) for half an hour. The orange-red solution was diluted with water (50 ml) filtered off and the filtrate was extracted thrice with small amount of amylalcohol. The anthocyanidis was transferred in 1% aqueous hydrochloric acid solution with excess of Petroleum ether (40-60°). The acid layer was washed with light petrol and with become repeatedly, excess of concentrated hydrochloric acid was added in it, when a deep crimson coloured solid(aglycone) crystallised out. The filtrate obtained after removal of the aglycone was neutralised with barium carbonate, filtered and concentrated under manufacture of a syrapy mass.

ZGEI

IV. 27 CHROMATOGRAPHY OF AGLYCONE

The purity of the aglycone was checked on whatman No.1 filter paper when a single spot was observed in each case using following solvent systems:

- (i) Acetic acid— concentrated hydrochloric acid Nater (30:3:10 v/v) system gg

These RE values, corresponding to the Pelargenidin 75.

IV 38 IDENTIFICATION OF SUGAR

reduced the Fehling's solution and it formed an osazone having mopo190° when treated with phenyl hydrazine reagent. The rf value of
the sugar was 0.18 in n-Sutanol section stater (4:1:5) v/v) system,
which corresponds to Deglucose. The identity of the sugar was further
confirmed by the co-chromatography with an authentic sample.

Potassium permanganate oxidation

The glycoside(F),(30 mg) was reflected with aqueous potassium permanganate solution (20 ml) on a water-bath for four hours. The reaction mixture was cooled and excess of manganese dioxide removed by adding sodium bisulphite to it. The reaction mixture them was extracted with other and it was shaken well with saturated aqueous sodium bicarbonate. This was acidified with hydrochloric acid and then extracted with ether. On concentration the other extract gave p-hydroxy benzoic acid, which confirmed by mixed m.p. and paper co-chromatography with an authentic sample. The purified p-hydroxy benzoic acid had m.p. 210 C, (Lit. m.p. 213 C)⁵⁶.

The rf value 0.86 in butanoltpyridine aqueous sodium chloride (1:1:2) saturated system 76, which corresponded to paydroxy bensoic acid.

IV.39 PERICHATE CHIDATION

The glycoside (30 mg), was dissolved in(20 ml) alidehyde free ethanol (90%) and to it saturated sodium metaperiodate solution (20 ml) in same ethanol was added and made upto 50 ml solution in a measuring flash. After 40 hours, (5 ml) aliquots were taken out from the reaction mixtures. The periodate consumption was estimated by titrating against standard hypo solution and liberated formic acid by titrating against standardised sodium hydroxide solution due to the method of Jones etal \$1. Molecular weight of the compound (7) = 468.70.

For 5 ml of the solution

0.011N sodium thiosulphate(hypo) consumed 2.3 ml and 0.011 sodium hydroxide consumed 2.5 ml 0.56 ml for each mole of the glycoside moles of formic acid liberated 2.1 moles of periodate consumed 2.1

IV.40 ENZYMIC HYDROLMS IS

The glycoside(20 mg) was dissolved in aqueous ethanol(20 ml) and emulsin solution (25 ml) was added and the mixture was kept at moom temperature for four days. Then the mixture was extracted with amyl alcohal. The aqueous layer was concentrated to a syrup. The paper chromatography of syrup in n-butanol:acetic acidsWater (4:1:5 v/v) revealed the presence of a single spot, R_g 0.18, corresponding to glucose.

TV-41 ABSORPTION SPECTRUM AND VISIBLE SPECTRA OF THE

All the measurment were taken using Beckmann model 17 , 17 , spectrophotometer and 1% methanolic hydrochloric acid as medium.

Solution and reagent		max(na)	shift.
(A) GENCOSIDE (F)			
(1) Atmethanolie hydrochlor	513		
(11)Austhanolic hydrochloric		523	***
(B) AGLYCOIE mas	of the	Mest on Alc13	addition of solution
(1) B+Mg-OH-Hell 89	520	No sh	if¢.
	530	No sh	ift

IV.42 IR SPECTRUM OF COMPOUND (F)

The following prominent peaks (cm⁻¹) were observed in the IR spectrum of the glycoside:

1620 cm⁻¹, 1550 cm⁻¹, 1500 cm⁻¹, 1440 cm⁻¹, 1380cm⁻¹, 1287 cm⁻¹, 845-820 cm⁻¹

- Duthie, J.F., Flora of the Upper Gangetic Plain and of the Adjacent Siwalik and Sub-Himalyan tracts, Vol. 1, P.367, (1960), Copyright by Government of India.
- 2. Chopra, R.N. and I.C., and Nayer S.L. 'Glossary of Indian Medicinal Plants', Page No. 123 (1956).
- 3. Howes, F.N. 'A Dictionary of useful Everyday Plants and their common names', Page No. 81, (1974).
- 4. James Y.P., Chen. U.S. 3,067, 103 Dec.4, 1962, Appl.July 22,(1960)
- 5. Kako Hongha K.K. Jpn. Nokai Tokyo Koho 80, 108, 464, 20, Aug. (1980), Appl. 79 116, 456, 15 Feb. (1979).
- 6. Kim, Gang Man. Sumyu Konghak, Holji, 13 (3), 129-32(1976)(Rogean).
- 7. Mikami, Yoichiro, Yajima, Izuma, Izumi (Hasagawa, T., Co.,(Ikd.)

 Jpn. Kokai, Tokyo Koho 79 96,532 (cl.0098 61/00)31 July.1979,

 Appl.78/21 648,1 7 Jan.(1978).
- 8. Yu. Ju-Hyun., Yoo, Seung-Kon., Yang, Ryung, Hanguk Sikpum Mwahakhoe chi(1975), 7 (1), 30-6 (Eng.).
- 9. Asai, L.T. and Nakamara, M. Sot Mag. Tokyo 33 , 70-1(1919).
- 10. Manesada, L.T., J. Pharm, Sac. Japan. No. 486, 666-71(1922).
- 11. Rozo Hayashir Proc. Esp. Acad. (Tokyo) 20 , 311-17 (1944) (Gers.).
- 12. Makino, Tokeda, Yoshio; Nishimura, Hiroshi, Mudota, Gamu; Inouye, Hiroyuki; Chem. Pharm. Bull, 26 (11), 2644-6 (1976) (Eng.).
- Inouye, Hiroyuki; Takeda, Yoshio, Shaito, Satsuo; Nishimura,
 Hiroshi, Sakuragi, Ryako; Yakugaka Zasshi, 94(5),577-86(1974)(Japan.
- 14. Veda, Shinichis Kobayashi, Moji,s Muramatsu, Takeharus Inouye, Hiroyuki (Fac. Pharm. Sci., Kyoto Univ. Kyoto, Japan), Planta. Med., 41(2), 186-91, (1981), (Sng.).
- Matsumoto, Mitsuo, Vehara, Yusaku, Japan. Kokai 7829, 965,
 Mar (1978), Appl. 76/103, 963, 31 Aug. (1976).

- 16. Rozo, Hayashi, Tachiko Isaka, and Gen Suzushi, Misc. Rapts.
 Research, Inst. Nat. Resources No.17-18, 33-42 (1950).
- 17. Inouye, Hiroyuki; Takeda, Yoshio; Wishimura, Hiroshi.
 Phytochem., 11(10), 2219-26, (1974) (Eng.).
- 18. Endo, Tohru; Taguchi, Meihachiro, Chem. Pharm. Bull, 21(12), 2684-8 (1973), (Eng.).
- 19. Inouye, Hiroyuki, Saito, Setsuo, Tguchi, Heihachiro, Shdo, Tohru, Tetrahedron Lett., 28, 2347-50 (1969) (Germ.).
- 20. Kasuhara, Nobuo; Suzuki, Shinji; Shioda, Asao.. Japan Kokai, (1976), Appl. 74/143, OS9, 14 Dec. (1974).
- 21. Inouye, Hiroyuki, Saito, Setsuo, Shingu, Tetsuro, Tetrahedron Lett., (41), 3581-4, (1970) (Gara)
- 22. Ando, Tohru; Taguchi, Heihachire; Chem. Pharm. Bull., 18(5), 1066-7 (1979) (Eng.).
- 23. Yang-chi, Chun, Yao Hsuen Hsuch Pao, 11 (5), 342-5(1964).
- 24. Chatterjee, Mrs. A., Saha, S.K.; Battacharya, S., (Hep. Pure. Chem. Univ. Call. Sci., Calcutta, 700009 India, Indian J.Chem., Sect. B. 19 H(5), 421-2 (1980) (Ang.).
- 25. Chhabra, S.C., Gupta, S.R., Sharma, N.D. (Dep. Chem. Univ. Delhi, Delhi, Endia), Phytochem. 16(3), 399 (1977) (Eng.).
- 26. Krishmamurti, M., Seshadri, Tiruvekkata, R., Sharma, N.D., Indian, J.Chem. 9(2), 189-90 (1971), (Eng.).
- 27. Rao, A.V., Rama; Venkataraman, Krishnaswami, Chakrabarti, P.; Sanyal, A.K.; Bose, P.K., Indian J. Chem., 8(5), 398-400(1970)(Enc
- 28. Mamari, Durga, Gupta, S.R. Sharma, N.D., Indian J. Chem. Sect.S. 17(8 2) 181-2 (1979) (Eng.).
- 29. Dutes, M.K., Ganguly, J.N., and Shattacharys, A.N., J. Indian.
 Chem. 43 (5), 380 (1966) (Ang.).
- 30. Reddy, G.G.S., Rangaswami, S., Sandar, R., (Dep. Chem. Univ. Dolhi, Delhi India), Planta Mad., 32 (3),206-11 (1977) (Eng.)

- 31. Joshi, Krishna, C., Singh. P., Pardasani, R.T., (Dep. Chem. Univ. Sajasthan, Jaipur, India), J. Endian, Chem. Soc. <u>56</u> (3), 327-8 (1979) (Sng.).
- 32. Forster, M.O., and Kashaviah, Assath Narain Rao, J. Chem. Soc., 127, 2176, (1925) (Eng.).
- 33. Raddy, G.C.S. Ayangar, K.N.N., Rangaswami, S., Phytochem., 12(7), 1831 (1973) (Eng.).
- 34. Qunatilaka, A.A., Leslie, Sirimanne, Sarath, R., Sotheeswaran, Subramanianm, Nakanishi, Tsufosa, J.Chem.Res.Synop.[7].
 216-17 (1979) (ang.).
- 35. Delaude, C., Kapundu, Mpusa Ball. Soc, R. Sci., Liege, 44-56, 493-4 (1975) (Fr.).
- 36. Govindachari, Tuticorin R., Jadhav, S.J., Joshi, Balwant, S., Kamal, Venkatesh. N., Mohamed, P.A., Parthesarathy, P.C., Patankar, S.J., Frakashi, D., Rano, D.F., Viswanathan, N., India, J.Chem. Z (3), 308-10 (1979)(Eng.).
- 37. Raddy, G.C.S., Ayengar, K.N.M., Rangaswami, S., Phytochem., 14 (1), 307 (1975), (Eng.).
- 38. Raddy, G.C.S., Ayengar, K.N.M., Ramgaswami, S., India J.Chem., 13 (7), 749-50 (1975) (Sng.).
- 39. Aidia Ochrolenca and Aidiamicrantha, Delaude, C., Bull, Soc.R. Sci., Liege, 43 (3/4) 257-9(1974) (Fr.).
- 40. J. Pharmacol. 4, 64-8, (1954) f., Hold 3, 1(1953).
- 41. Aburada, Masaki,; Sasaki, Hiroshi, Harada, Masatoshi, Yakugaku Sasshi, 96(2), 147-53 (1976), (Japan).
- 42. Darwis Amar and A. Grevenstukl. Geneeskuna Tijdschar.Nederland India.76, 1948-84 (1936).
- 43. Shinoda, J.; J. Pharm. Soc. (Japan), 48, 214 (1928).
- 44. Marti, V.V.S., Rajagopalan, S. and Row, L.R., Proc. Rad. Acad. Sci. 34 , 319-23 (1951).

- 45. Asahina, Y; and Inubuse, M; Ber., 61, 1946 (1928).
- 46. Briggs, L.H. and Locker, R.H., J. Chem. Soc., 3136 (1951).
- 47. Geissman, T-A-; "Modern Methods of Plant Analysis" ed by Peach, K. and Tracey, M.V. Springer-Verlag, Berlin, Vol. III, 450 (1955).
- 48. Venkstaraman, K, In 'Progress in the chemistry of Organic Natural Products' 86. by Zeichmeister, California Institute of Technology, Padasona, Vol. 17.
- 49. Suendsen, A.B.; Pharm. Acta. Melv; 34, 9 (1959).
- 50. Asahina, Y and Inpuse, My Ber. 64 1256 (1931).
- \$1. Jurd. L. and Hogowitz, R.M., J. Org. Cham., 22 1618 (1957).
- 52. Jurd. Les 'The chemistry of Flavonoids compounds', ed. by T.A. Geissman, Pergamon Press, Orford, P. 107 (1962).
- 53. Nordstron, C.G. and Smain, T., J.Chem. Soc., 2764 , (1953).
- 54. Manafield, G.H., Swain, T and Nordstron, C.G., Mature, 172, 23(1953)
- 55. Willstaller, R. and Mallison, H., Anan, 408, 40 (1915).
- 56. Dimorth, O. and Paust, T., Ber., 54 , 3030, (1921).
- 57. Horhammer, L. and Hensel, R.; Arch. Pharm. Berl., 286, 425 (1953); 288, 315 (1955).
- 58. Wilson, C.W., "A study of the Boric Acid colour Reactions of Playene Perivatives", J. Weer. Chem. Soc., 61, 2303, (1939).
- 59. Horowits, R.M., J. Am. Chem. Sec. . 72, 6561, (1957).
- 60. Swain, T., Chem. and Ind., 1480, (1954).
- 61. Hillis, W.E. and Vrbach, G., Nature, 182, 657. £1958).
- 62. George, T.B., Daugles, Ç.D. and Wander, S.M., Arnal. Chem., 23 , 1982 (1951).
- 63. Wisenberger, Mikeo Chemic., 22 . 51. (1947).
- 64. Balcher, R. and Godbert, A.L.; In semi-micro Casantitative organic Analysis', P. 164, Longmann-Green and Co., New York, I End 6d(1954).

- 65. Belcher, R., Fildes, J.E. and Nutten, A.J., Analytical Chem. Acta. 12, 16 (1955).
- 66. Shimizu, M., J.Pharm. Soc. (Japan), 1339.71 . (1957).
- 67. Douglus, C.D., Morris, Q.L. and Wender, S.H. J. Amer. Chem. Soc. 4023, 73 (1951).
- 68. Horowitz, R.M., J. Org. Chem. 1733, 22, (1957).
- 69.(a) Deam, P.M., In "Naturally Occuring Oxygenring Compound", P. 288, Butterworths, London (1963).
 - (b) Wilson, C.W., J.Amer. Chem. Soc., 2303, 61 (1939).
- 70. Hill, R.D. and Maskins, G.D., J. Chem. Soc. 760 (1958).
- 71. Bell, J.V., Heisler, J., Tann-embaum, H. and Goldenson, J., Anal. Chem. 1720, <u>25</u> (1953).
- 72. Ory. H.A., Anal. Chem., 509, 32, (1960).
- 73. Wagner, H., In *Methods in Poly Phenol Chemistry*, edited by J.B. Pridham, P. 38, Pergamon Press, Octord, (1964).
- 74. Shaw, B.L and Simpson, T.H., J.Chem. Soc., 5027, (1952).
- 75. Jard, L. and Hogowitz, R.M., J. Org. cham. 1395, 21 (1956).
- 76. Feighl, F. In 'Spot tests in Organic chemistry', Elesevier Publishing Company, London (1960) F. 466.
- 77. Gage, T.B., Doughlass, C.D. and Wender, S.A., Anal. Chem. 1958, 23 , (1951).
- 78. Jurd, L., Arch. Bio. Chem. Bio Phys., 376, 63 , (1956).
- 79. Hough, L., J. Chem. Soc., 1702, (1950).
- 80. Ayako, M. and Shunisada, Nippon Negai Kagaku Kaishi, Japan, 317, 39 (8), (1965).
- 81. Jurd, L. and Rolle, L.A., J. Amer. Chem. Soc., 5227,89(1958).
- 82. Segal, L. O' Connor, R.T. and Eggerton, F.V., J. Amar. Cham. Sec., 2807, 82 , (1960).
- 83. Bunicet, J.C. and Badger, R.M., J. Amer. Chem. Soc, 4397.72(1950).

- 84. Helbron, I. and Babury, H.H. In Dictionary of Organic Compounds*, Vol. II, P. 259,
- 85. Hirst, S.L. and Jones, J.K.N. & Che m. Soc., 1659 (1949).
- 96. Richter, D. J. Chem. Soc., 1702 (1950).
- 87. Simpson, T.H. and Garden, L., J. Chem. Soc., 4638, (1952).
- 88. Jurd, L., J. Amer. Chem. Soc., 80 , 5531, (1958).
- 89. Horhammer, L. and Hansel, R., Arch. Pharm., Ber. 37, 287, (1954).